XI. *The Growth of the Oocyte in Antedon: a Morphological Study in the Cell-Metabolism.*

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[Plates 29–31.]

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General Introduction.

THE present paper is an attempt to interpret, in terms of the cell-metabolism, the morphological changes exhibited by the actively secreting cell under natural conditions. For this purpose I have selected the ovarian egg. In no other cell are the structural changes more evident or their apparent correlation with the cell-activity more striking. The definite limits to the period of secretive activity and the fact that the products of secretion are stored up in the cell enable us to compare the appearances presented by the cell-structures at varying and easily recognisable phases of the cell-activity. Further, we can compare eggs in which a considerable quantity of yolk is formed unaided with those which form but little or have it partially formed for them. Finally, we are able in many cases to follow the gradual divergence from a common stock and structure of nutritive cells and definitive ova. Not only are we thus provided with exceptionally favourable opportunities for a comparative study of the egg under very different, but perfectly definite, physiological conditions, but the structural changes accompanying these conditions—the growth and varying form of the germinal vesicle, the behaviour of the chromatin and nucleolus, the appearance of the yolk nucleus and of yolk formation—all these constitute a wealth of morphological evidence as to the relative significance of the various cell-structures in the cell-metabolism.

That this evidence has not as yet sufficed to enable us to come to any agreement as to even the most general physiological significance of the cell-structures in the cell-metabolism* is, I believe, due not so much to insufficiency of data as to the

* Our ignorance of the relative significance of cell-structures in the cell-metabolism is sufficiently clearly indicated by the following conflicting theories taken from the recent literature on the subject. Their number could be multiplied well-nigh indefinitely :—The structural features of the germinal vesicle stage, *e.g.*, the subdivision of the chromatin, are directly correlated with great metabolic activity (RÜCKERT, 1892, BORN, 1894); are not related to the cell-metabolism, but are due to other factors, either retention in the ovary (HÄCKER, 1893 and 1895), or necessity for the nourishment of the hereditary

manner in which these data have been studied; the behaviour of individual cell-structures is considered apart from the metabolism of the cell as a whole, and their changes in form are explained by the assumption of inconceivable structural complexity, while obvious physical factors are ignored.*

In the present investigation I have confined myself to the study of the egg in one type, *Antedon bifida*, PENNANT, and I have only referred to the observations of previous workers on other types when these appeared to have a direct and unmistakable bearing on my own observations on Antedon. It will be my endeavour in subsequent papers to determine how far the conclusions in the present paper are supported by the behaviour of the egg in other types.†

Material and Methods.—The material employed was obtained at Plymouth, originally in the spring of 1903, during a vacation tenancy of the Zoological Society's Table. Since then I have had additional material sent up from time to time as the development of the work rendered necessary.

In all cases the material was studied in section, the entire pinnules being fixed and then decalcified in a solution containing nitric acid and phloroglucin. After fixation with sublimate acetic or bichromate acetic, however, this subsequent decalcification was not needed.

I believe that the varying behaviour of the several cell-structures with varying fixation provides a surer basis for their subsequent differentiation than do subtle colour reactions to complex staining mixtures. I have, therefore, in the present investigation used only one stain—HEIDENHAIN'S iron-alum hæmatoxylin, with or without a plasma stain. On the other hand, I have made use of several fixing reagents, my choice of these being determined by the desire to obtain as wide

qualities (LUBOSCH, 1902). The chromosomes persist throughout the germinal vesicle stage (RÜCKERT, 1892, BORN, 1894, R. HERTWIG, 1902); the whole of the chromatin is massed into one or more nucleoli (CARNOY and LEBRUN, 1897, HARTMANN, 1902). The nucleolus is a waste bye-product of chromatin activity (HÄCKER, 1893 and 1895); it supplies nutriment to the chromatin (FLEMING, 1882, BAMBEKE, 1885, KORSCHELT, 1895, LUBOSCH, 1902); it is the organ by which the unorganised chromatin of the cytoplasm is converted into the organised chromatin of the nucleus (R. HERTWIG, 1902): it discharges into the cytoplasm material by means of which yolk formation takes place (MACALLUM, 1891, JORDAN, 1893). The yolk nucleus is the formative centre for the yolk (MERTENS, 1895, BAMBEKE, 1898); it is a mere condensation of nutritive material in the cytoplasm (LUBOSCH, 1892); it is derived from, or is analogous to, the centrosome (BALBIANI, 1893, MEVES, 1894, FOOT, 1896, MUNSON, 1898).

* HOFMEISTER, 1901, 'Die chemische Organisation der Zelle' (p. 23), "Denn man verkennt oder unterschätzt den Einfluss, den die Zusammensetzung einer Lösung auf die Formen darin sich gestaltender Gebilde ausübt."

† The need for a revision of existing descriptions of the egg's growth before attempting to bring this process, for all eggs, under one general scheme of metabolism is forcibly indicated by the fact that some of the features in which many other eggs appear to differ from Antedon as now described have been described by previous workers for this very type. Among these may be mentioned the fragmentation of the yolk nucleus and assumed active participation of the latter structure in yolk formation, so also the amœboid movements of the germinal vesicle and the radiations from the latter into the cytoplasm.

a range of variation as possible in the appearance of the fixed material. The reagents thus selected for the purpose of the present investigation are five in number, viz. :— Saturated sublimate solution; saturated sublimate with 5 per cent. acetic acid; potassium bichromate 3-per-cent.-solution with 5 per cent. acetic acid; HERMANN'S fluid; and a solution containing potassium bichromate, sodium sulphite, nitric and osmic acids, and which for convenience of description will be referred to in the paper as "BNO." This solution, which was recommended to me by Dr. ALLEN, of Plymouth, gave—for the tissues of Antedon at all events—a very poor general fixation, but rendered a useful and most unexpected service in differentiating certain regions of the nucleolus.

Special Introduction: General Structure of the Ovary in Antedon.

The ovaries of Antedon lie in the proximal portions of the pinnules, and cause a considerable swelling of the latter during the season of sexual maturity. They are connected with one another in the arm by a genital rachis which arises ontogenetically as an outgrowth of the axial organ in the calyx.* The genital rachis lies between the subtentacular and cœlomic canals, and consists of a solid cord of cells which, in the pinnular extensions of the rachis, become the ova. Both ovary and genital rachis are enclosed by a flattened epithelium, which, however, does not function as a germinal epithelium. A sinus surrounds the ovary or rachis and its limiting epithelium.† In the immature individuals the ovaries are represented merely by the unexpanded pinnular terminations of the genital rachis, and, like the latter, are filled with closely packed oogonia. Prior to the seasonal maturity these oogonia are found in active mitosis, and this proliferation, together with the subsequent growth of the resulting oocytes, speedily produces the enlarged ovarial terminations of the genital rachis of the mature individual.‡

In the maturing ovary the growing oocytes form a more or less solid mass of cells, between which oogonia are still present in considerable numbers. The only constant feature in the arrangement of the germ-cells consists, as HAMANN pointed out, in the invariably peripheral position of the still growing oocyte. As there is no germinal epithelium, the new generations of oocytes being derived from the scattered oogonia, there must either be an active peripheral migration of the oocyte prior to the commencement of its growth, or else only those oocytes which happen to already possess this position ever commence the latter process. The fact that this position

* For an account of the development of the axial organ and references to previous work in Antedon development, see O. SEELIGER, 1892 ('Zool. Jahrb.,' Abt. Anat. u. Ont., vol. 6).

† HAMANN, 1889 ('Jen. Zeitsch. f. Naturwiss.,' vol. 23, p. 344), could not determine the connection of this sinus with those in the calyx, though its vascular nature in other echinoderms is undoubted.

‡ For a detailed account of the structure of the ovary in the adult Antedon, see O. HAMANN, 1889 ('Jen. Zeitsch. f. Naturwiss.,' vol. 23, p. 343). In this paper HAMANN corrects the original account of LUDWIG, 1877, describing a germinal epithelium and follicular arrangement of the germ-cells similar to that occurring in Holothurians.

should be maintained during the "growth" period of the egg, and that it should be frequently lost while yolk formation is still in progress, is of great interest in connection with the question of the relation of yolk formation—that is the actual appearance of the definitive yolk spherules—to the general cell-metabolism. In the maturer individuals the scattered oogonia are only rarely found in mitosis,* while in the mature pinnules these abortive oogonia have undergone considerable degenerative changes, the details of which I have not attempted to follow in the present paper.†

THE OOGONIUM.

When freely floating (Plate 29, fig. 1) the oogonia are amœboid in form, but when closely packed their outline is not always distinguishable. The nucleus is approximately spherical and measures from 3 μ to 5 μ across. There is a well-marked nuclear membrane, and the chromatin is in the form of irregular and deeply staining masses at the nodal points of a clearly defined linin reticulum. In many nuclei the meshes of this reticulum are so large that the greater part of the chromatin forms a series of peripheral masses on the nuclear membrane, while the remainder is massed around the nucleolus, the linin being reduced to a few strands radiating out from the surface of the nucleolus to the peripherally placed chromatin masses (fig. 1). There is a single nucleolus ranging from 1 μ to nearly 2 μ in size, and apparently always peripheral in position. After Hermann fixation with sufficient differentiation, or after the use of the fixing solution "BNO," the nucleolus appears as a pale-brown or yellow perfectly regular and sharply defined spherule surrounded by the deeply staining chromatin. In some cases, especially in the very immature pinnules, there are not only two nucleoli, but the nucleus itself is distinctly double (fig. 2).‡

In the dividing oogonia the nucleus measures 5 μ to 6 μ, and the cell body 7 μ to

* *Cf.* also HAMANN, 1889 (pp. 346 and 347).

† These abortive ova appear to constitute the "yolk cells" of CUÉNOT and others. CUÉNOT, 1892 ("Notes sur les Echinodermes," 'Zool. Anz.,' vol. 15, p. 121), states that he has seen a relation between the egg and these "yolk cells" in Antedon similar to that seen in ophiuroids and in the asteroid *Cridella*, and which was long ago described by J. MULLER and LUDWIG (1874); the cytoplasm of the egg being continued through the vitelline membrane as a peduncle which penetrates into the "yolk cells." The nearest approach I have ever seen to such an arrangement is that shown in fig. 40, which represents an egg in the later phases of yolk formation. At one portion of its surface the vitelline membrane appears to be interrupted, and a small group of cells, similar to the numerous undifferentiated germ-cells to be found among the growing ova, appears to be in continuity with the cytoplasm of the egg.

Whatever may be the explanation of this appearance, it is certain that it is far too rare to constitute a normal stage in yolk formation; moreover, it never occurs during the growth period of the oocyte, nor even at an earlier stage in yolk formation, which would hardly be the case if the appearance had any significance in the normal nutrition of the egg.

‡ *Cf.* the similar double nuclei described for the germ-cells of various copepods in the papers of RÜCKERT and HÄCKER (1895), and for several other types in subsequent papers (*e.g.*, "Torpedo," RÜCKERT, 1899). *Cf.* especially CONKLIN'S account (1901) of this condition in Crepidula.

8 μ, thus showing a considerable increase in size on that of the resting oogonia.* A split spireme thread is formed (fig. 3), and this enters a synapsis stage, becoming aggregated around the nucleolus on one side of the nucleus (figs. 4 and 5). During this process the thread shortens and thickens, so that the longitudinal split usually entirely disappears, but the thread character of the chromatin is always evident. In the resolution of the synapsis the nucleolus is seen to be still present, of normal size, deeply stained, and sharply spherical, while the chromatin appears to radiate out from it on to the nuclear membrane in the form of (apparently) eight deeply stained and homogeneous strands (fig. 6). The latter lose their apparent connection with the nucleolus and, without further segmentation, give rise to the (apparently) eight U-shaped chromosomes (figs. 7 and 8), which leave the nuclear membrane and aggregate together in the centre of the nucleus to form the equatorial plate (fig. 9). From this stage onwards it is not possible to detect the nucleolus, nor is there any trace of this structure in the completed spindle (fig. 10).

THE OOCYTE.

I. *The Chromatin and Nucleolus in the Young Oocyte.*

In spite of the large number of oogonia found in active mitosis, I have never seen the anaphase stages of these oogonial divisions, and I have therefore been unable to trace the actual formation of the oocyte.

In what appears, however, to be the stage immediately succeeding the completion of the last oogonial division, the young oocyte possesses a distinct nuclear membrane enclosing a spherical nucleus measuring 5 μ in diameter, in the centre of which the nucleolus is suspended by a faintly stained linin reticulum (fig. 11). The nucleolus measures 1·25 μ in diameter, and is, after most fixing reagents, deeply stained. As in the oogonia, however, fixation with the "BNO" solution, or considerable differentiation after fixation with Hermann, leave the nucleolus faintly stained; and it is then seen to be surrounded, as in the oogonia, by a layer of chromatin.† In the oocyte, however, though the whole of the chromatin present is massed around the nucleolus, it forms a layer too thin to appreciably affect the spherical form of the latter structure. Fig. 17 shows the appearance of an oocyte at a somewhat later stage, after fixation with HERMANN's fluid and after considerable extraction.

The absence of anaphase stages has made it impossible to determine the part taken by the massive chromosomes of the oogonium in the formation of the nucleus of the oocyte; and therefore also the mode of origin of the nucleolus. But having regard to the behaviour of the nucleolus in the oogonium—the rarity with which it is seen during the formation of the equatorial plate and its invariable absence in the

* *Cf.* MUNSON's account (1898) of a similar growth of the germ-cell prior to mitosis in Limulus.

† VAN BAMBEKE, 1898 (p. 517), describes a similar enclosure of the nucleolus by (here only some of) the chromatin in the young oocyte of Pholcus. (*Cf.* also HEIDENHAIN, 1892.)

completed spindle—it seems fairly certain that this structure arises in the oocyte as a new formation, and is not genetically related to that of the oogonium.

The invariably central position of the rapidly growing nucleolus of the young oocyte conclusively shows that the peripheral position of this structure in the relatively quiescent oogonium cannot have any significance as denoting an extra-nuclear origin for this structure.

The young oocyte grows rapidly, and with the growth of the cell-body there is a corresponding growth of the nucleus and nucleolus. Early in this process the nuclear reticulum, at first faintly stained, commences to stain more deeply. This increase in the staining capacity of the nuclear reticulum proceeds from the nucleolus outwards, and though the nuclear reticulum can, with suitable illumination, be seen to really extend from the nucleolus out to the nuclear membrane, the process gives it the appearance of consisting of longer or shorter, more or less deeply staining threads, radiating out from the surface of the nucleolus (figs. 12 and 13). Sooner or later, but always by the time the oocyte reaches the size shown in fig. 16, the basophile reaction has extended over the whole of the nuclear reticulum. Soon after this the strands lose their connection with the surface of the nucleolus and with the nuclear membrane and become irregularly scattered over the nucleus (fig. 18), an arrangement which is maintained throughout the remaining history of the oocyte, the irregularly scattered chromatin strands showing no further spatial relation either to the nucleolus or to the nuclear membrane.

II. *The Chromatin in the Growing Oocyte.*

As the chromatin threads lose their radial arrangement they become more deeply stained and sharply defined, and now, for the first time, bear distinct, though as yet small, deeply stained spherules (figs. 16 and 18). Often the strands appear to be composed solely of these minute spherules; sometimes, however, appearances suggest that the latter are really arranged on a fine, deeply stained and apparently homogeneous axial thread, the zigzag and branched character of which gives to the chromatin strands a "mossy" or "filagree" appearance. During the period preceding the commencement of yolk formation, and thus during the progressive increase in the basophile reaction of the cytoplasm (*vide infra*), the chromatin is always relatively small in quantity, and is often very faintly stained. As yolk formation progresses, however, both the quantity and the staining capacity of the chromatin greatly increase, and at the same time the spherules on the chromatin threads grow in size until, in the adult egg, they may equal the spherules of the yolk (*e.g.*, figs. 25, 62, 63, 64, all of which are from the same individual and fixed with sublimate acetic).

The staining capacity of these "chromatin spherules" and the size they ultimately attain varies considerably in different individuals (*e.g.*, figs. 65 and 66) and is also to some extent dependent on the mode of fixation, and in material fixed solely with

sublimate solution there is no trace of either chromatin or chromatin spherules. This variable behaviour of the chromatin, as will be seen later, is of considerable importance in enabling us to clearly distinguish between nucleolar and chromatin spherules. On the completion of yolk formation the condition of the chromatin remains unchanged for as long as the egg remains in the pinnule.

III. *The Nucleolus in the Growing Oocyte.*

(a) *General Appearance and Staining Reactions of the Nucleolus.*—In the younger oocytes the nucleolus, which is, at this stage, invariably central in position, is homogeneous in appearance and, after most fixing reagents, deeply basophile. As growth proceeds, however, this uniform staining reaction is lost, the nucleolus showing a tendency to part with the basic stain during extraction. This de-staining is always from within outwards and may, with moderate extraction, be so extensive as to include the whole nucleolus. More usually, however, there is a considerable cortical region which tenaciously retains the basic stain. The readiness with which this de-staining takes place is mainly determined by the condition of the nucleolus itself at the moment of fixation and is independent of the age of the egg; eggs of the same age and in the same ovary differing greatly in this respect. After fixation with the "BNO" solution the general staining reactions of the nucleolus are almost exactly reversed, the peripheral portion having a faintly yellow, horn-like appearance, while the region corresponding to the de-stained area of other material shows, where it abuts on the cortical region, a distinct tendency to retain the basic stain; so that in the younger oocytes, in which the greater part of the nucleolus lies within the thickness of the section, the de-stained central area, when this is present, appears in this material deeply basophile and surrounded by a clear transparent cortical region. This condition is shown in figs. 43 and 44. When there is no "de-stained area" in "BNO" material the whole nucleolus appears of a faintly yellow, horn-like substance.

The more faintly staining internal region of the nucleolus is never spherical in form, and its size, though mainly due to the condition of the nucleolus itself, is, as we have seen, partly determined by the extent of extraction the slide has undergone; moreover, its contents are distinctly acidophile, far more so than is the caryolymph. It is evident, therefore, that the faintly staining area of the nucleolus is not of the nature of a vacuole.

True vacuoles, spherical in form and with clear unstained contents, do, however, occur in the nucleolus. Their occurrence, like that of the de-stained area, is quite inconstant, for they are sometimes present, sometimes absent, at all stages of the egg's growth, though they are far more rarely met with in the earlier than in later stages. They are always much smaller than the faintly staining area, and although they may be present throughout the whole body of the nucleolus when this is

deeply basophile (fig. 52), they are almost or quite confined to the periphery when a considerable de-stained area is present (fig. 50).

(b) *The Discharge of Spherules from the Nucleolus.*—The nucleolar vacuoles, even when they reach a considerable size, never cause the slightest projection on the surface of the nucleolus, nor have I ever seen any other indication that they discharge their contents to the exterior. The appearance of the faintly staining region, on the other hand, is frequently such as to suggest that a discharge from it into the caryolymph is taking place. Thus in the nucleoli shown in figs. 48 and 49, wherever the faintly staining area comes to the surface it causes a slight but distinct projection. In no case, however, do appearances ever suggest that the acidophile substance of the faintly staining area is itself discharged.

In the adult egg an extensive faintly staining area is always present, and its form and position almost invariably suggest that a discharge is taking place from it, a suggestion which is greatly intensified by the almost invariable presence in the caryolymph of deeply stained spherules, so placed as to appear as if recently discharged as a stream from that region of the nucleolar surface to which the faintly staining area most closely approaches (figs. 55, 56, etc.). Occasionally the faintly staining area approaches the surface of the nucleolus at more than one point; in such cases it is common to find spherules in the caryolymph in relation to each such point (figs. 52 and 60). It is only rarely that such spherules are found in the nucleus which do not show this spatial relation to the nucleolus; and when such spherules do occur, they are always more or less isolated in position and few in number.

The mode of origin of these spherules as a discharge from the faintly staining area of the nucleolus, which their arrangement irresistibly suggests, is still further indicated by the appearance presented by the nuclei shown in figs. 52 and 53. In each of these a spherule, similar to those in the caryolymph, is seen in the centre, not of the de-stained area, but of one of the vacuoles in the cortical region. These figures are taken from material fixed with sublimate, in which the cortical region of the nucleolus is always as deeply stained as the discharged spherules. A still more striking appearance is presented by fig. 54, which is taken from Hermann material, in which, in the adult egg, the basophile substance of the nucleolus is relatively easily de-stained to a faint brown. The figure shows a deeply basophile spherule, identical with those in the caryolymph immediately outside the faintly staining area of the nucleolus, but lying in a vacuole within the substance of the cortical region, and, by reason of its intensely basophile character, standing in striking contrast to it.

These spherules, like the substance of the nucleolus itself, are equally well fixed with all reagents, and, like the cortical substance of the nucleolus, are nearly always deeply stained. Their staining reactions, however, do not exactly correspond with those of the cortical substance; for when, as we have just seen frequently happens in the older eggs after Hermann fixation, the cortical substance of the nucleolus is de-stained to a faint brown, the discharged spherules in the same egg are usually

deeply basophile. In size these nucleolar spherules often equal the "peripheral spherules," with which they also agree in showing a much greater range of variation than do the yolk spheres proper (*vide infra*).

Similar spherules, presenting the appearance of having been recently discharged from the nucleolus, are also occasionally, though much more rarely, to be found in the growing oocyte. Fig. 24 shows a stage prior to the commencement of yolk formation, in which a group of deeply basophile spherules, almost as large as those seen in the adult egg, stand in direct relation to the peripheral and slightly projecting de-stained area of the nucleolus. Fig. 18 is taken from material in which the nucleolus, as also the chromatin, shows a much stronger basophile affinity; it represents a still younger egg, in which a stream of spherules is in the act of being discharged from the nucleolus, though the latter is so intensely basophile that extraction has not sufficed to differentiate a less intensely basophile internal region. The special interest of this section lies in the fact that, owing to the eccentric position of the nucleolus and the outward direction of the discharge, the stream of spherules already lies well in the cytoplasm, while still retaining its obvious spatial relation to the nucleolus.

The fact that this appearance of a discharge of spherules from the nucleolus should be so rarely seen in the growing oocyte admits of very simple explanation. In the growing egg the intermittent discharges must be separated from one another by a considerable interval,* while the time occupied by the actual discharge must be very brief. The chances, therefore, against the moment of discharge coinciding with that of fixation are very great. In the adult egg, on the other hand, the great frequency with which the discharge is seen cannot be explained as due to its more frequent occurrence, for this would indicate a degree of activity on the part of the nucleolus at this stage for which there is no particle of evidence. As will be shown later (p. 457), the completion of yolk formation is accompanied by the complete cessation of all nucleolar activity and growth, the nucleolus remaining of the same size and retaining the same appearance for as long as the egg remains in the pinnule.

There appear to me to be only two possible explanations: either the discharge so frequently seen in the adult egg represents the last which occurred during the waning activity of the nucleolus and which has since remained unchanged, or, and I think this the more probable explanation, the discharge in the adult egg is caused by the contraction of the nucleolus at the moment of fixation, the activity of the nucleolus during the closing period of the egg's growth being insufficient to provide the necessary pressure for a normal discharge of the gradually accumulating material. I have, however, never seen the appearance of a discharge of spherules from the nucleolus of the still growing oocyte after yolk formation has commenced. I am unable to do more than suggest that this restriction of the contracting influence of

* HÄCKER, 1893 (p. 283), describes an interval of four to eight hours between successive discharges from the central vacuole of the nucleolus in the living sea-urchin egg.

the fixing reagent to the nucleolus of the adult egg may be due to a change in the physical properties of the corticle layers of the nucleolus after the activity of the latter structure has ceased.

(c) *Fate of the Discharged Nucleolar Spherules.*—The occurrence of discharged nucleolar spherules in the young oocyte long before there is any obvious indication of the formation of spherules on the chromatin threads (*e.g.*, fig. 24), their almost invariable spatial relation to the de-stained area of the nucleolus, and their uniform behaviour, irrespective of the nature of the fixing reagent (*e.g.*, figs. 54, 55, 57, etc.), sufficiently clearly distinguish these spherules from those on the chromatin threads. The fact that the nucleolar spherules are less frequently met with in later than in earlier stages indicates that they do not accumulate as such within the nucleus. Equally certain is it that they do not dissolve in the caryolymph, for there is never, at any period, any trace of nucleolar spherules in course of solution in the nucleus, although, if such a process occurred, it would necessarily occupy some time. There remains, therefore, as the only possible fate for the discharged nucleolar spherules, an immediate passage to the cytoplasm. This cytoplasmic fate for the nucleolar spherules has been already suggested by fig. 18 and considerable additional evidence to the same end will be adduced in considering the origin of the yolk nucleus (p. 466).

IV. *The Nucleolus in the Adult Egg.*

In addition to the persistence in the caryolymph of the last nucleolar discharge, the completion of yolk formation is marked by another phenomenon in connection with the nucleolus, namely, the gradual formation, on the surface of this structure, of one or more lens-shaped accumulations. At its first appearance this accumulation is extremely thin, tapering away imperceptibly at its edge on the nucleolar surface (figs. 59, 60, 61). It gradually increases in size, becoming more and more hemispherical as it does so, until it may equal more than one-third the total bulk of the nucleolus (fig. 58). Its staining affinity generally resembles that of the de-stained area of the nucleolus, being usually weakly basophile, but showing considerable affinity for the acid stain. The substance of the "accumulation" is always homogeneous in its staining reactions; it never shows a de-stained area, and it is never vacuolated. The absence of any connection between nucleolar activity and the formation of the "accumulation" is indicated by the complete absence of any spatial relation between this structure and either the discharged spherules in the nucleus or the de-stained area within the nucleolus. Moreover, the simultaneous appearance of the accumulation and completion of yolk formation is also accompanied by a complete cessation of the growth of the nucleolus, a growth which, as already noted, shows at earlier stages a remarkably constant relation to that of the egg. The inactivity of the nucleolus during the formation of the accumulation is further shown by the fact that nucleoli with a large amount of this accumulated material on their surface

and, therefore, presumably fixed some considerable time after the completion of yolk formation, do not appear to possess less of the basophile cortical substance than do those in which the accumulation is absent.

V. *Constitution of the Nucleolus in Antedon.*

The approximately spherical form of the nucleolus and its steady growth, irrespective of the facility with which it parts with the basic stain, indicate that the whole of the nucleolus is formed of a ground substance continuous alike through the basophile and acidophile regions. The fact that the occasional departures from the spherical form are invariably produced by that portion of the nucleolar surface in contact with the de-stained area (figs. 24, 49, 51, &c.), together with the fact that it is from this region the nucleolar discharge invariably takes place, indicate that the capacity for tenaciously retaining the basic stain is accompanied by a considerably firmer consistency of this portion of the nucleolar substance. As the loss of this reaction proceeds from within outwards, the outermost layers of the deeply basophile cortex must possess a considerably firmer consistency than those lying further in. This condition readily explains the entire absence of any indication of a discharge taking place directly to the exterior from the vacuoles in the cortical region, however peripherally they may be placed, and lends support to the assumption that the vacuoles discharge instead into the more fluid substance of the faintly staining area, an assumption which is still further suggested by the periodical discharge taking place from the latter.

The products of the activity of the nucleolus, the formation of which is accompanied by the appearance of vacuoles in the basophile region and the loss of this basophile reaction, accumulate, therefore, within the nucleolus, the internal pressure of which at the same time steadily increases.* When this internal pressure reaches a certain point, the firmer cortex of the nucleolus is ruptured at the point of least resistance, that is, at that point on its surface to which the more fluid faintly staining area happens to approach most closely. When this rupture of the cortex occurs, the accumulated products of nucleolar activity, partly in the form of a clear fluid, partly as basophile spherules, are discharged from all parts of the nucleolus through the less resisting, more faintly staining, internal region to the exterior.

I have not been able to ascertain that the nucleoli accompanied by an obvious discharge are any smaller than those in eggs of a similar age which are not so accompanied; but the general contraction of the nucleolus necessary to cause this discharge would be so slight that the resulting decrease in diameter would fall well within the limits of variation of individual nucleoli.

The accumulation of products of nucleolar activity which must be present within the nucleolus prior to its discharge is not very evident. The fluid portion is probably

* Note HÄCKER'S suggestion that the nucleolus constitutes an osmotic system (1893, p. 290).

contained within the frequently numerous vacuoles in the cortical region of the nucleolus. Only very rarely, however, are spherules, similar to those discharged, to be seen within the nucleolus. This is to be expected, for the spherular form of the discharged material is as much due to the fluid character of the surrounding caryolymph as to the physical consistency of the discharged material itself, and when this is formed within the nucleolus, it is surrounded by the relatively firm nucleolar substance, and does not, therefore, assume the spherular form.* In all those rare cases, already described, in which I have seen spherules within the nucleolus similar to those outside, they have each been contained within a vacuole, and were therefore here also surrounded by a fluid medium. In the great majority of cases we should expect the basophile substance resulting from the nucleolar activity to form an irregular zone on the inner side of the as yet unaltered cortical region, between this and the faintly staining area, and only to assume a spherular form as it is discharged into the more fluid caryolymph. There is some evidence to show that this is the actual mode of accumulation of the substance of the future spherules. As already noted, the whole nucleolus of the adult egg in Hermann material readily de-stains to a faint brown, but this is not the case in the younger eggs, in which only the internal region de-stains, the cortical region remaining intensely basophile. In the older, but still actively growing egg, appearances similar to that shown in fig. 46 are to be sometimes seen; the cortical region, instead of being deeply basophile, is stained brown, and only a zone between this and the faintly stained internal region retains the basic stain. When we remember that the spherules, whether lying in the caryolymph, or stranded in a vacuole in the adult nucleolus, are deeply basophile, while in Hermann material the whole of the cortical region of the (at this stage) inactive nucleolus can usually be readily de-stained to a relatively faint brown, it seems almost certain that the deeply stained zone shown in fig. 46 represents accumulated material destined to constitute a spherular discharge. The appearance of the nucleoli in the "BNO" material already referred to strikingly supports this suggestion. In this material, in the actively growing oocytes, the cortical region of the nucleolus always remains clear and unstained, the basic stain only being retained by the zone between the cortex and the de-stained area, and when the latter is absent the whole nucleolus remains unstained (figs. 43, 44, 45).

It has already been shown that the extent of the faintly staining area shows no gradual increase in size with the growth of the egg; eggs of the same age and in the same pinnule possessing nucleoli sometimes deeply stained and sometimes greatly de-stained, even in the same section. On the other hand, the growth in bulk of the nucleolus does proceed perfectly steadily, and shows a striking correlation with the growth of the egg. It is evident, therefore, that while the faintly staining ground substance of the nucleolus steadily increases in bulk, the amount of basophile

* See also p. 464, *re* non-spherular discharges from the nucleolus.

substance present in the nucleolus fluctuates greatly, sometimes gaining, sometimes losing, on the nucleolar activity.

Since it is only in the earlier stages of its growth that the nucleolus shows any spatial relation to the chromatin, and only very exceptionally at any period shows any relation to the nuclear membrane, it follows that the caryolymph must be the "immediate" source for both these nucleolar substances.

This source for the basophile substance is very clearly indicated by the appearance often seen in material in which the nucleolus readily parts with the basic stain, the faintly stained nucleolus being studded all over with minute basophile areas, each flush with the nucleolar surface externally, and projecting irregularly into the faintly stained ground substance of the nucleolus internally (fig. 47). This appearance would seem to suggest that the basophile substance enters the nucleolus by diffusion through the nucleolar surface. Osmotic diffusion through the surface layers of the nucleolus is also suggested by the mechanism of discharge already described (p. 458).

The caryolymph as the immediate source for the ground substance of the nucleolus is convincingly shown by the formation of the nucleolar accumulation on the completion of yolk formation, for, as we have seen, this accumulation not only strongly resembles the faintly staining area of the nucleolus in its staining reactions, but its formation synchronises with the cessation of growth on the part of the nucleolus. It is impossible, therefore, to avoid the conclusion that this accumulation represents material to which the steady increase in size of the nucleolus was formerly due, and which, now that the nucleolar activity has ceased, can no longer be incorporated in the body of the nucleolus.

The formation of the nucleolar accumulations on the cessation of nucleolar activity would seem to suggest that, in so far as this nucleolar constituent is concerned, the mode of growth of the nucleolus in earlier stages is by passive deposition rather than by osmotic diffusion.

VI. *Significance of the Nucleolus in Antedon.*

So little evidence has been forthcoming in the present investigation to show how the nucleolus first arises, that we are deprived of whatever indication its mode of origin might have provided as to the significance of this structure in the cell-metabolism. There are, however, two points concerning the young nucleolus to which attention may be directed. The first point concerns its position. In the resting oogonium the nucleolus appears to be always peripheral in position, whereas in the actively growing oocyte it is for some time invariably central, a fact which scarcely points to the peripheral position of the nucleolus as denoting a cytoplasmic origin for this structure.*

* MONTGOMERY, 1898 (p. 523), after giving several instances of young nucleoli showing an invariably peripheral position, concludes that "the nucleolar substance in many, if not in all, cases has an extranuclear origin."

The second point concerns the close relation which the young nucleolus invariably shows to the chromatin. Both the peripherally placed nucleolus of the resting oogonia and the centrally placed nucleolus of the actively growing oocyte are surrounded by a portion at least of the chromatin. In the oogonium this relation is maintained until preparation for mitosis leads to the withdrawal of the perinucleolar chromatin. In the young oocyte the gradual subdivision and more equal distribution of the chromatin which accompanies the increasing metabolism of the growing cell more speedily leads to the same result. The fact that in the young oocyte, at a stage which can be but very little removed from the anaphase stages of the preceding oogonial division, there should already be a relatively large nucleolus surrounded by a thin layer representing the whole of the chromatin of the cell emphasises the importance of this relation between the nucleolus and the chromatin. It is evident, however, that though this arrangement may facilitate, it cannot be essential to, the formation of the nucleolar substance, for in the growing oocyte the nucleolus more than trebles its diameter after it has lost all direct relation with the chromatin.

The later behaviour of the nucleolus in the oocyte of Antedon offers, on the other hand, considerable evidence of a highly suggestive character relative to the significance of this structure in the cell-metabolism. One of the most striking morphological features which the nucleolus invariably presents is its spherical or approximately spherical form, a form in which minimal surface area is associated with maximal bulk. In this respect the nucleolus offers an instructive contrast to the chromatin, where there is an obvious endeavour to assume a form with as great a superficial area as possible. It is impossible not to conclude that this difference between the nucleolus and chromatin indicates the nature of the difference in their physiological significance; that while the chromatin is active, and probably selectively so, taking what it requires from the caryolymph, the nucleolus, at least in so far as getting fresh material is concerned, is passive, the nucleolar constituents being deposited on, or diffusing through, its surface without any effort on the part of the nucleolus itself. We have already seen that the further changes which the nucleolar substance undergoes take place most actively within the interior of the nucleolus, and give no indication that a reaction between the nucleolar substance and the caryolymph is essential to the process.

Although the immediate source for both the nucleolar constituents must be the caryolymph, yet their very different behaviour when nucleolar activity ceases would seem to indicate their derivation from distinct ultimate sources. There are three possible sources for these nucleolar constituents, viz., the chromatin, the cytoplasm, and the nutritive plasma of the parent organism by which the egg is bathed.

(1) *Origin of the Ground Substance of the Nucleolus.*—Apart from the *a priori* improbability of the nutritive plasma exercising a selective action in depositing food material on to the nucleolus, this source for the ground substance of the nucleolus

is excluded by the remarkably constant relation which the size of the nucleolus always shows to the stage of the egg's growth, a relation very difficult to explain if the nucleolus passively receives the bulk of its substance directly from a source outside the cell. On the other hand, the cytoplasm is equally excluded as a source for the ground substance of the nucleolus, for this substance continues to be deposited on to the surface of the nucleolus after the completion of yolk formation, at a time, therefore, when practically the whole of the cytoplasm has been converted into metabolically inactive yolk spherules. There remains, therefore, only the chromatin as the source for the ground substance of the nucleolus, a source already indicated by the intimate relation this structure shows to the nucleolus in the young oocyte, in which the as yet relatively inactive metabolism permits of a massed arrangement of the chromatin.

(2) *Origin of the Basophile Substance of the Nucleolus.*—The origin of the basophile constituent of the nucleolus presents more difficulty. We have seen that there is reason to believe the activity of the chromatin to be continued for some time at least after the simultaneous completion of yolk formation and cessation of nucleolar activity. The only structure, therefore, which in its behaviour shows a direct temporal relation to the cessation of nucleolar activity is the cytoplasm. On the completion of yolk formation there is not only an abrupt cessation of nucleolar activity, but there is also a sudden failure in the supply of the basophile nucleolar constituent, a fact which certainly points to the cytoplasm as the source for this substance.

It is not, however, only the completion of yolk formation, but also the complete cessation of all cytoplasmic activity, which coincides with the termination of nucleolar activity. As we have already seen, the production of the basophile nucleolar substance shows no relation to the commencement of yolk formation, but is produced continuously throughout the growth of the egg. This substance must be the result, therefore, not of yolk formation itself, but of a much more fundamental vital process of the cytoplasm, a conclusion which is supported by the fact that we already have, in the "peripheral spherules" presently to be described, an obvious by-product of the actual process of yolk formation.*

That the nucleolus deals with the waste products of cytoplasmic activity rather than with the nutritive material in the caryolymph is also indicated by the ultimate fate of the discharged material described below.

The nucleolus of Antedon would thus appear to be derived from the chromatin, and to function as the excretory organ of the cell, the waste products of cytoplasmic activity undergoing their final changes within its substance and being discharged, now, presumably, inert and harmless, into the cytoplasm, where they slowly dissolve away.

* See also p. 482, *re* relation of yolk formation to the general cell-metabolism, and the reference to MUNSON'S observations on the Limulus egg (p. 484).

VII. *Some Previous Observations on the Nucleolus.*

It is not within the scope of the present paper to review the conflicting theories which have been propounded in recent years as to the significance of the nucleolus. The theory which most closely approaches the one I have adopted above is that of HÄCKER (1893 and 1895), by which the nucleolar substance is regarded as a by-product of chromatin activity, a waste product in so far as the nucleus is concerned. Within the nucleolus this substance is assumed to undergo further changes, producing on the one hand the firmer peripheral layer, and on the other the substance of the vacuoles, these latter being either periodically or finally discharged. It will be noticed that in this theory HÄCKER regards the firm crust of the nucleolus as one of the by-products of the nucleolar activity, whereas I interpret it as the most recently deposited, and as yet unaltered, nucleolar substance. A more important difference, however, between HÄCKER's conception and my own lies in the fact that HÄCKER recognises only one source—the chromatin—for the nucleolar material. As a result of this difference HÄCKER associates cessation of nucleolar activity with commencing concentration of the chromatin, and not with the cessation of cytoplasmic activity. He, therefore, interprets the decreased staining capacity of the later-formed nucleolar material as indicating a gradual change in the chemical nature of the waste products of the chromatin during its period of waning activity.

HÄCKER (1893, p. 291) describes for the living Echinus ovum the appearance of the nucleolar material as weakly basophile spherules which, after circling round the nucleolus, fuse with it. That HÄCKER thus makes the nucleolar substance unite with the nucleolus in the form of spherules does not constitute a very important difference from the condition I have described for Antedon, for the manner in which this substance is deposited must be determined mainly by the relation its physical constitution bears to that of the caryolymph, a relation which may very well be supposed to vary within considerable limits in the eggs of different animals.

MONTGOMERY (1898) describes for the ova of Montagua a similar union of spherules with the nucleolus, but he believes them to be yolk spherules taken up by the nucleus from the cytoplasm. MONTGOMERY reasons that since the yolk in the cytoplasm is undoubtedly nutritive in function, so the spherules absorbed by the nucleolus must also be destined for nutritive purposes, that is, for the nutrition of the germinal vesicle!

MONTGOMERY imagines the "nutritive globules" thus absorbed by the nucleolus to constitute the vacuoles of the latter, and denies that these arise from the nucleolar substance itself. This is an altogether exceptional view, and the formation of vacuoles and their discharge have been so frequently described for the nucleolus, and even seen in the living egg, that their formation within the nucleolus and

subsequent discharge from the latter must be accepted as well-established phenomena.*

In many cases deeply staining bodies, "nucleolini," have been described as being present in, and even discharged from, the nucleolus.† While in the nucleolus these are usually described as lying within the vacuoles of the nucleolus. LUBOSCH (1903)‡ describes the discharge, not only of the "nucleolinus," but of the surrounding clear area also. The description refers, however, to the extremely variable behaviour of the small nucleoli of the Triton egg. In the case of "Hauptnucleoli," it is common to find the discharge described as leaving behind a flask-shaped depression, a description very difficult to harmonise with the assumption of a fluid or semi-fluid consistency for the nucleolus, but perfectly intelligible if the interpretation be accepted which I have given for the somewhat similar appearance seen in Antedon.§

I have suggested above that the form in which the nucleolar substance is conveyed to this structure, whether as spherules or by deposition directly upon the nucleolar surface, would probably depend entirely on the relative physical characters of this substance and the caryolymph. I am strongly inclined to think that a similar explanation would account for the formation of those enigmatical band-like structures so often described as emanating from the nucleolus,|| the relative physical consistency of the caryolymph and discharged nucleolar substance not necessitating the assumption by the latter of a spherular form. In this connection it is interesting to note that LUBOSCH, in confirming CARNOY's account of the formation of these structures in Triton, describes the occasional occurrence of "Kapsel-nukleolen," which frequently recall the appearance seen in Antedon, the nucleolus containing a faintly stained area, often apparently continuous at one point with the caryolymph, and in which are deeply-stained spherules. This observation tends to show that even in the same animal the discharge may be either band-like or spherular.

In admitting that the material discharged from the nucleolus may pass directly to the cytoplasm, HÄCKER (1893) suggests that certain inpushings of the nuclear membrane may indicate a relation between the cytoplasm and the discharge from the

* For an account of observations on the discharge of vacuoles from the nucleolus, see HÄCKER, 1893 (p. 282).

† For numerous references to descriptions of nucleolini, see MONTGOMERY, 1898 (p. 509).

‡ 'Jen. Zeitsch. f. Naturwiss.,' vol. 37. Compare this author's figs. 6, 8, and 9 with figs. 52, 53, and 54 in the present paper.

§ *Cf.* BALBIANI, 1883: Phalangium, and especially MUNSON'S account of the Limulus egg (1898, fig. 110 and p. 159). Note also Schneider's description of the origin of small nucleoli in Klossia from within the Hauptnucleolus and their passage outwards through a "canal micropylar." *Cf.* also a similar origin for the smaller nucleoli in *Gregorina blattarium*, described by Marshall, 1892.

|| *E.g.*, RÜCKERT, 1894, confirming HÄCKER, 1893, for copepods. So also LUBOSCH, 1903, confirming CARNOY, 1897, for Triton.

nucleolus, and recalls BALBIANI's funnel in the germinal vesicle of Geophilus (1883). In the very numerous cases in which I have seen the appearance of a discharge taking place from the nucleolus there has never been the slightest indication of such a behaviour on the part of the cytoplasm, nor, on the interpretation of nucleolar activity which I have given, should we expect such a relation to exist.

There is still considerable difference of opinion on this question of an outward passage from the nucleolus into the cytoplasm of formed elements, such as I have described as taking place in Antedon. LUBOSCH, in his review of 1902 (p. 773), after referring to and criticising the existing descriptions of such an occurrence, concludes that there is as yet no single case in which it has been proved to occur. MONTGOMERY (1898, p. 524) also reviews previous descriptions of an outward migration of nucleolar substance, and describes himself, though in a most unconvincing manner, the discharge into the cytoplasm "of all but one" of the 15 nucleoli in the nucleus of the subcuticular gland-cells of Piscicola (see p. 489).

VIII. *The Yolk Nucleus: Its Mode of Origin.*

In the preceding section (Behaviour of the Nucleolus) it was shown that throughout the growth of the oocyte, the nucleolus periodically discharges deeply basophile spherules into the caryolymph. It was also shown that, as there was no accumulation of these spherules in the nucleus, nor any sign of their absorption, either by the caryolymph or by the chromatin, it was probable, from evidence provided by the nucleus alone, that these spherules pass at once into the cytoplasm.

In the present section additional evidence, provided by the cytoplasm, will be adduced for this outward passage of formed spherules from the nucleus; and it will be shown that during the earlier stages of the growth of the oocyte these discharged "nucleolar" spherules slowly accumulate in the cytoplasm until they give rise to the definitive yolk nucleus.

(a) *The Basophile Spherules in the Cytoplasm.*—Basophile spherules commence to appear in the cytoplasm even before the chromatin has lost its radial relation to the nucleolus (figs. 13, 14, 15, 17, 19); and they occur in increasing numbers as growth proceeds, until the formation of the definitive yolk nucleus, in eggs with a nucleolus of from $7\,\mu$ to $8\,\mu$ (figs. 22 and 23).* The spherules are found with all fixing reagents, and are always deeply basophile, even after prolonged extraction. The individual spherules are sharply defined, and vary in size from $0{\cdot}5\,\mu$ to $1{\cdot}5\,\mu$, or even occasionally $2\,\mu$. They do not increase in size with the growth of the egg, the few

* KORSCHELT, 1889, figures and briefly describes refringent granules surrounding the germinal vesicle of the younger oocytes of Antedon, and which are still more numerous at later stages. He compares these to the granules formed by the nutritive cells of Dytiscus, which, he assumes, pass into the cytoplasm of the oocyte. He makes no reference to the yolk nucleus of Antedon.

spherules first to appear in the young oocyte being as large as the more numerous spherules at later stages, and very small spherules are rare at any stage. The spherules are only occasionally found isolated in the cytoplasm; far more usually they are associated together in small clusters of from 3 to 6, irregularly disposed around the nucleus, and lying either in actual contact with the nuclear membrane or at some little distance from it. They are seldom, however, found far from the nucleus, and are rarely, if ever, found peripheral in position. The spherules of any one cluster vary in size within the limits already noted, and it is rare to find a group consisting solely of very small spherules.

(b) *Identity of these Spherules with those Discharged from the Nucleolus.*—It has already been noted that, compared with the period intervening between successive discharges, the time actually occupied by the spherules in passing from the nucleolus into the cytoplasm must be very brief, and that this is ample explanation for the rarity with which this process is seen. The few instances in which this passage outwards has coincided with the moment of fixation in the still growing oocyte have already been referred to in considering the behaviour of the nucleolus (p. 456, figs. 18 and 24). In fig. 24 the definitive yolk nucleus, to be described below, is already present, and in addition to this, three groups of basophile spherules lie in the cytoplasm in the plane of section, and are obviously similar in all respects with the group of spherules which, as already noticed, is to be seen in the act of being discharged from the nucleolus. Fig. 15 shows a much younger oocyte, in which the chromatin is still radially arranged. There is a large group of basophile spherules to the right, while on the top of the figure four or five scattered spherules are shown, one of which can only with difficulty be seen to be in the cytoplasm and not in the nucleus. Another spherule is actually in the nucleus, but showing a very obvious spatial relation to the spherules in the cytoplasm.

In addition to the spatial relation which the nucleolar spherules are thus seen to frequently bear to those in the cytoplasm, the nucleolar origin of the latter is also indicated by their arrangement in the cytoplasm. It is difficult on any other supposition to account for their arrangement in small groups, irregularly scattered round the nucleus; and this arrangement, together with the fact that they are never found in a peripheral position, and that there are, at this stage of the egg's growth, no cells in relation to the egg's periphery, exclude the possibility of an extracellular origin. The fact that the spherules are of much the same size in the youngest as in older oocytes, and that groups of very small spherules are rarely, if ever, seen, exclude both a cytoplasmic origin and a mode of origin by diffusion through the nuclear membrane.

It should also be noted that the groups in the cytoplasm show no relation to the distal ends of the radially-arranged chromatin strands, and moreover continue to appear after this arrangement of the chromatin has been lost.

The basophile spherules, therefore, which are discharged at intervals in batches

from the nucleolus, pass at once into the cytoplasm, and in the young oocyte form scattered groups around the nucleus.*

(c) *Formation of the Definitive Yolk Nucleus.*—In the young oocyte, the nucleolar spherules in the cytoplasm increase in number with every discharge from the nucleolus, but otherwise remain unchanged. The cytoplasm also at this period shows no other change than a considerable increase in bulk, and a very slight increase in basophile staining reaction. Sooner or later, however, and always by the time the nucleolus measures 8 μ, in eggs, therefore, of from 20 μ to 30 μ in diameter, a portion of the hitherto uniformly staining cytoplasm commences to retain the basic stain. This more deeply staining region of the cytoplasm is at first extremely irregular and diffuse in character, and nearly always stands in relation to some, at least, of the groups of nucleolar spherules, and the appearance thus presented strongly suggests that a diffusion is taking place of the deeply staining substance of the nucleolar spherules on to the surrounding cytoplasm. This condition is shown in fig. 21. In fig. 22, which represents a still later stage, the more deeply staining cytoplasm nearly surrounds the nucleus, and thus stands in relation to all the groups of nucleolar spherules in the cytoplasm. During this diffusion, the sharply marked and deeply stained nucleolar spherules become more and more scarce, and at the same time the more deeply staining region of the cytoplasm gradually loses its irregular and scattered condition, becoming instead compact and regular, and assumes the form of a faintly staining, but clearly defined, concavo-convex, lens-shaped body, closely applied to, though never in actual contact with, the surface of the spherical nucleus, and measuring 25 μ to 30 μ in diameter and from 8 μ to 10 μ in thickness. This is the definitive yolk nucleus (figs. 25, 28, 29).

That the more deeply staining area of the cytoplasm, and consequently also the definitive yolk nucleus, does not arise by a simple aggregation of the nucleolar spherules is shown by the difference in staining reaction of the yolk nucleus and of the nucleolar spherules. Fig. 22 represents an egg taken from sublimate material, and shows two groups of nucleolar spherules which have only just come into relation with the stainable area; their deeply basophile character and sharp outline offer a striking contrast to the somewhat diffuse and less deeply stained spherules of the more deeply staining cytoplasmic area. This contrast is still more striking with material fixed with other reagents; thus with Hermann, or with acetic bichromate, the nucleolar spherules are—as with sublimate—deeply stained and clearly defined, while the texture of the yolk-nuclear area, like that of the rest of the cytoplasm with these reagents, is finely granulo-reticular in character, only differing from the surrounding cytoplasm in its greater affinity for the basic stain (fig. 25).

Although the accumulated nucleolar spherules in the cytoplasm greatly diminish in number during the formation of the yolk nucleus, and may even be entirely

* For references to previous descriptions of nucleolar substance passing into the cytoplasm, see, MONTGOMERY, 1898 (p. 524).

absent on the completion of this process, they nevertheless are still occasionally to be seen in eggs in which a definitive yolk nucleus is present. Thus, in fig. 24, not only is the nucleolus in the act of discharging a group of spherules similar to the groups already in the cytoplasm, but there is also a group closely applied to the inner side of the yolk nucleus. The rapid increase in staining capacity shown by the yolk nucleus soon after its appearance, and which is described below, is in all probability to be explained by this incorporation of additional nucleolar spherules. At later stages, as the nucleolar discharges become more and more infrequent, spherules in the cytoplasm are more rarely met with, and are never found in contact with the yolk nucleus after this has lost its intensely basophile character, although even in the adult egg groups of partially dissolved nucleolar spherules are occasionally to be seen embedded in the yolks (fig. 65).

IX. *The Definitive Yolk Nucleus.*

(a) *Changes in Form, Position, and Staining Capacity.*—When the stainable area first assumes the regular form of the definitive yolk nucleus, it is, as already noted, much less deeply basophile than the original nucleolar spherules from which it has been derived. This faintly staining condition of the yolk nucleus does not last long, and in eggs very slightly, if at all, larger, the yolk nucleus is as deeply stained as the original spherules. Accompanying this increase in staining capacity on the part of the yolk nucleus there is a corresponding, though slower and much less strongly marked, increase in the basophile reaction of the cytoplasm.

That the basophile reaction of the yolk nucleus is, in the main at least, due to other causes than those determining the corresponding reaction of the cytoplasm has been already suggested by the mode of origin of the yolk nucleus. It is still further indicated by considerable differences in this staining reaction in these two regions of the cytoplasm; for not only does the yolk nucleus stain deeply before there is any basophile reaction on the part of the general cytoplasm, but the basophile reaction of the yolk nucleus is only very slightly influenced by the character of the fixing reagent, while that of the cytoplasm varies greatly, and with some reagents, *e.g.*, acetic bichromate, may be entirely absent. Moreover, even in material in which the basophile reaction of the cytoplasm is most marked, extraction always greatly de-stains the latter without appreciably affecting the appearance of the yolk nucleus.

As the yolk nucleus acquires its deeply basophile character it moves away from the surface of the nucleus, becoming rounder and shorter as it does so, and assumes a position midway between the nucleus and the egg-periphery (figs. 30 and 34). At this stage the yolk nucleus and, after sublimate fixation, the cytoplasm also, has reached its maximum basophile reaction. Yolk formation now commences, and very soon after this, while yolk formation is still only faintly indicated, the yolk nucleus assumes a peripheral position close to, though not as a rule in actual contact with,

the egg-periphery. At the same time it loses its regular form and rounded contour, acquiring instead a form roughly determined by the nature of the egg-periphery, and a somewhat irregular outline. At this period, and while yolk formation is as yet mainly confined to the egg-periphery, the yolk nucleus shows a considerable increase in size and accompanying loss of staining capacity. As yolk formation proceeds, the yolk nucleus retains its peripheral position, and appears to spread out over the ever-increasing surface of the egg, becoming considerably thinner as it does so, always, however, covering the same proportional area of the egg-periphery. Fig. 32 shows the commencement of this process, the yolk nucleus is still deeply stained, though peripheral in position, and already measures $45\,\mu$ by only $6\,\mu$ in thickness. Fig. 33 shows a very slightly later stage in yolk formation, the nucleolus still measuring $14\,\mu$, but the yolk nucleus now forms an irregular and faintly staining disc, flattened out against, though not in actual contact with, the egg-periphery, and measuring $100\,\mu$ in diameter by only $3\,\mu$ in thickness. A similar stage is shown in figs. 37 and 38. Beyond this point, as yolk formation approaches completion, the progressive loss of staining capacity of the yolk nucleus which has accompanied yolk formation results in its total disappearance. Fig. 40 represents an egg in which yolk formation is well in hand, and in which the yolk nucleus forms a relatively small, faintly stained, and somewhat irregular disc-shaped area, measuring only $25\,\mu$ by $5\,\mu$. A similar stage is seen in figs. 41 and 42.

Neither during its disappearance nor during the earlier stages of yolk formation does the yolk nucleus ever show the slightest indication of undergoing a granular disintegration. Its outline, though never showing the even character it presented prior to the commencement of yolk formation, is never so irregular as to be indefinite, and as soon as the loss of staining capacity has proceeded sufficiently far it is seen that the structure of the yolk nucleus in all respects resembles that of the surrounding yolk ;* the only difference being the ever-decreasing basophile reaction, and it is evident that yolk formation has proceeded in the region of the cytoplasm constituting the "yolk nucleus" exactly as in other regions of the cell (figs. 38 and 67). During its actual disappearance the yolk nucleus is frequently represented by several detached areas ; but in all such cases these regions are so placed with reference to one another as to indicate that they are not detached fragments, but persisting regions of the original yolk nucleus, the unequal disappearance of the last traces of the basophile reaction having led to their isolation. Very shortly after this stage the yolk nucleus disappears from view altogether, and no trace of it is ever to be seen on the completion of yolk formation.

(b) *Significance of the "Clear Space" accompanying the Yolk Nucleus.*—In the

* *Cf.* CRETY (*loc. cit.*, fig. 14 and p. 266), in which the remains of the yolk nucleus of an adult (?) egg are shown as a homogeneous or finely punctate area, from which radiate out the strands of the cytoplasmic reticulum.

above description of the behaviour of the yolk nucleus no mention has been made of a peculiar feature in connection with this structure which is of very frequent occurrence. After most fixing reagents, the yolk nucleus is accompanied, usually on its outer side, by a clear space in the cytoplasm. On the first appearance of the definitive yolk nucleus, while this is still faintly staining, this space is absent, the cytoplasm in the immediate neighbourhood of the yolk nucleus in no way differing from the rest. This condition is maintained throughout the subsequent history of the yolk nucleus in material fixed solely in a saturated solution of sublimate; but after most other fixing reagents, *e.g.*, sublimate and acetic, bichromate and acetic, Hermann's fluid, etc., a clear area appears on the outer side of the yolk nucleus and grows step by step as the latter increases in staining capacity, and only finally disappears shortly before the disappearance of the yolk nucleus itself, slowly decreasing in extent as the latter loses in staining capacity. Fig. 28 shows two adjacent eggs from material fixed with acetic bichromate. In the smaller egg there is a faintly stained yolk nucleus with little or no indication of a clear space. In the slightly larger egg the yolk nucleus, though of about the same size and shape, is deeply basophile, and is bounded on its outer surface by a large and fairly regular space which obviously contains no fixed elements. Fig. 29 shows a similar condition in material fixed in HERMANN'S fluid, and fig. 34 a very slightly later stage in sublimate acetic material. At later stages the clear space, like the yolk nucleus itself, is less regular in form, and though usually, or mainly, on the outer surface of the yolk nucleus, may occasionally completely surround it, or even be confined to its inner side, though this latter condition is rarely seen.

The gradual growth of the clear space, hand in hand with the increase in basophility of the yolk nucleus, and its disappearance with the decline of this reaction, *i.e.*, its coincidence with the period of apparent greatest activity of the yolk nucleus, seems at first sight to suggest that the clear area stands in some direct physiological relation to the activity of the yolk nucleus, and represents an accumulation of non-coagulable fluid in the living egg.* There are, however, several difficulties in the way of such an interpretation.

In the first place, the space is entirely absent in sublimate-fixed material (fig. 30), though present in material taken from the same individual, but fixed with an acid reagent (*e.g.*, compare figs. 27 and 29). The absence of the clear space in sublimate material cannot be due to the great coagulating power of this reagent having coagulated the fluid accumulation; for not only would such a well-marked segregation of material so different in constitution from the rest of the cytoplasm show some difference in staining capacity from the latter, a difference of which there is no trace (figs. 30 and 31), but there is frequently in this material no room for such an accumulation on the outside of the yolk nucleus (figs. 26, 27, 30, etc.). The clear

* Compare BAMBEKE'S interpretation of a similar space accompanying the yolk nucleus in the oocyte of *Pholcus*. (See also p. 475.)

space, therefore, is wholly unrepresented in sublimate material, and its presence in other material must be an artifact due to shrinkage during fixation. Indirectly the absence of the clear space in sublimate material is due to the greater capacity of this reagent for fixing simple proteids in the absence of a free acid; for the coagulation of these waste or metaplastic substances with which the yolk nucleus and general cytoplasm are loaded prevents the shrinkage which produces this space in other material. That this is the explanation of the absence of the clear space in sublimate material is very clearly shown by the fact that, even in acid fixed material, there is no clear space accompanying the yolk nucleus when this structure is faintly stained, and when it is, therefore, presumably less heavily loaded with non-coagulable material (figs. 28 and 42).

In the second place, both the clear space and the yolk nucleus of the older oocytes have lost the regular form they possessed in earlier stages, and it is then frequently seen that the outline of the clear space corresponds exactly with that of the yolk nucleus, a correspondence which can only be explained by supposing that the two surfaces were originally in contact and have been separated by shrinkage (fig. 38), and is wholly incompatible with the supposition that the space is caused by an accumulation of fluid material in the living egg. This interpretation of the space as a split due to shrinkage is also supported by the presence, on the surface of the yolk nucleus facing the space, of numerous frayed strands of cytoplasm (fig. 34). The extensive changes in the form and size of the yolk nucleus make it impossible to ascertain by direct measurements, on acid fixed and sublimate fixed material from the same individual, to what extent the shrinkage of the yolk nucleus is responsible for the clear space; but the fact that there is no great difference in size between yolk nuclei in oocytes of about the same age, the one accompanied, the other unaccompanied, by a clear space, shows that this contraction cannot be a very extensive one, and an explanation, other than the condition of the yolk nucleus, must be found for the very large clear space often present. The explanation is given by figs. 35 and 36 which clearly show that the contraction of the egg cytoplasm as a whole may often be largely responsible for augmenting the size of the clear area. In fig. 35 the yolk nucleus is accompanied by a clear space of a size, and in this particular case of a shape, wholly inconsistent with the assumption that it is due entirely, or even mainly, to the contraction of the yolk nucleus. In fig. 36 the size of the clear space is much more moderate, and the total area of the yolk nucleus and clear space in this example well accords with that of the unshrunken yolk nucleus in sublimate fixed oocytes of a similar age. In this case (*i.e.*, fig. 36) the general contraction of the cytoplasm has occured on the side of the egg remote from the yolk nucleus and shows that the clear area seen in fig. 35 is due to the combined contraction of both yolk nucleus and cytoplasm.

The clear space which often accompanies the yolk nucleus, usually on its outer side, is, therefore, an artifact, and is due to the shrinkage which results from the inability

of the acid fixing reagent to coagulate the waste and metaplastic substances with which the yolk nucleus and general cytoplasm are loaded. When the yolk nucleus is faintly stained, as on its first appearance and shortly before its disappearance, at times, therefore, when it is less heavily loaded with these substances, it is unaccompanied by a clear space in any material.

(c) *Significance of the Yolk Nucleus of Antedon in the Cell-Metabolism.*—It is not necessary to assume for the yolk nucleus an active participation in the cell-metabolism in order to explain the changes in form and position which this structure undergoes during yolk formation; for these changes are perfectly in accord with the assumption that they are entirely passive in character, and due to the gradual change in the physical consistency of the cytoplasm which precedes yolk formation, and to the great growth of the cytoplasm by which this latter process is accompanied.

As has been maintained elsewhere (p. 478), the progressively increasing basophile reaction of the cytoplasm which precedes yolk formation is due to the gradual loading of the cytoplasm with material supplied by the chromatin and passed out from the nucleus by diffusion through the nuclear membrane, the material so supplied being subsequently utilised in yolk formation. It is not unreasonable to suppose that this progressive change in the chemical constitution of the cytoplasm is accompanied also by a progressive change in its physical consistency, a change resulting either in an increased or decreased viscosity. The assumption that the change is in the direction of a more fluid consistency is the one which seems most easily correlated with the changes in the yolk nucleus immediately to be described.*

In the young egg the nucleolar spherules lie stationary and unchanged, slowly accumulating in the faintly staining cytoplasm, the viscid nature of which is indicated by the arrangement of the spherules in small clusters, each representing a discharge from the nucleolus, and each having penetrated only a little way into the cytoplasm. With the appearance of the basophile reaction and, as I have assumed, more fluid consistency of the cytoplasm, the nucleolar spherules can no longer retain their original character, and the substance of which they are composed diffuses on to the surrounding cytoplasm. At first the areas of the cytoplasm so affected are scattered and diffuse in character; gradually, however, they come together and assume a form in contact with the nucleus, which is entirely in agreement with the assumption that capillary laws constitute the sole morphogenic factor, the presence of the substance of the nucleolar spherules having so modified the physical character, and hence also the surface tension of the region of the cytoplasm which bears it, as to give it a "physical individuality" of its own.

By the continued change in the physical consistency of the cytoplasm which accompanies the increase in its basophile reaction, and by the yet further addition of

* Note also in this connection the tendency of the nucleus at this period to assume, with many fixing reagents, an irregular form (p. 487).

nucleolar substance to the yolk nucleus, the difference in surface tension between these two regions of the cytoplasm is still further augmented, and the yolk nucleus, leaving the surface of the nucleus, assumes a more compact and rounded form mid-way between the nucleus and the egg-periphery, often, indeed, being almost in contact with both.

Yolk formation now commences, and as this progresses, the coarsely alveolar structure it produces causes the cytoplasm to lose much of its original semi-fluid consistency. Capillary forces are, therefore, no longer able to exert the influence they formerly possessed in maintaining the regular shape of the yolk nucleus, and the smooth contour of the latter gives place to a somewhat irregular outline.

During the actual process of yolk formation there is a rapid and very considerable increase in the bulk of the cytoplasm, the diameter of the egg being often more than quadrupled during the process. As the yolk nucleus represents an area of the cytoplasm only differing from the rest by being infiltrated with the substance of the nucleolar spherules, it also should show an increase in bulk corresponding to that of the whole egg. This increase in the size of the yolk nucleus does actually occur in the earlier stages of yolk formation, but at a later stage it is masked by the great extension and thinning out of this structure at the egg-periphery. If the expansion of the cytoplasm during yolk formation were due to the equal and simultaneous expansion of all its parts, then the form and position of the yolk nucleus towards the close of yolk formation should be approximately similar to that seen in earlier stages, and some other cause than the simple increase in bulk of the cytoplasm would have to be found in order to account for the peripheral position and attenuated form which this structure invariably shows at this stage. But the increase in bulk of the cytoplasm, in other words yolk formation, as has already been shown, is not uniform all over the cytoplasm, but, apart from its first commencement, proceeds from without inwards. The increase in bulk thus caused in the peripheral layers of the cytoplasm, being unaccompanied by any great increase in the superficial area of the egg (since the expansion is confined for the present solely to the peripheral layers), results in a considerable increase in thickness and only a slight superficial extension of this portion of the yolk. It is at this stage that the yolk nucleus shows the increase in size already referred to. At a later stage, however, when yolk formation is general all over the interior of the egg, there is a very considerable increase in the size of the egg as a whole, but the peripheral layers of the yolk, having finished their expansion, can only keep pace with this increase in the superficial area of the egg by superficial extension. In the yolk nucleus we have a region of the yolk in which these changes can be actually observed, the thinning out and superficial extension which this structure shows being merely that to which the whole of the peripheral portion of the cytoplasm is at this period subjected. As we should expect from this process, the yolk nucleus approximately covers the same proportional area of the egg-surface at all stages of yolk formation.

There only remains to be explained why the yolk nucleus should always lie at the periphery of the cell at the commencement of yolk formation, for it is evident that it is primarily to this position that the changes in form described above are due. The assumption, however, that the outward migration of the yolk nucleus at the commencement of yolk formation is also passive, and due to general changes in the surrounding cytoplasm, does not offer much difficulty. During yolk formation we have a constant migration to the periphery of granules or droplets arising in the body of the cytoplasm. As it is impossible to assume that this outward migration is due to the activity of the droplets themselves, it must be concluded that there are forces in operation in the egg during yolk formation which tend to send formed bodies to the periphery. These forces may be of the nature of centrifugal diffusion currents, or, as perhaps is more probable, are simply the result of an effort on the part of the still metabolically active cytoplasm to remain in touch with the nucleus. In either case, the presence of the nucleolar substance on the yolk-nuclear area would serve to bring this structure under the influence of such forces, though the fact that the yolk nucleus is itself, in part, composed of ordinary cytoplasm would considerably weaken their action. This would explain why the yolk nucleus, though always more or less peripheral in position during yolk formation, is never quite so; it never competes with the "peripheral spherules" of the yolk already referred to and yet to be described for a position in actual contact with the vitelline membrane.

Thus the absence of any participation by the yolk nucleus of Antedon in the cell-metabolism is indicated both by the origin of this structure as a region of the cytoplasm on to which has diffused a portion only of the material intermittently discharged from the nucleolus throughout the growth of the oocyte, and by the fact that its subsequent behaviour can be entirely explained as due to the influence on a semi-fluid mass of the varying physical conditions obtaining in the cell.

The entirely fortuitous manner in which the yolk nucleus arises in Antedon offers a ready explanation of a hitherto puzzling fact, that though this structure is of constant occurrence in those forms in which it occurs at all, it is nevertheless frequently entirely absent in closely allied species. For it is obvious that very slight changes in the constitution of the yolk, or in the mode of its formation, might suffice to remove the conditions under which the diffusion described above could alone take place.

X. *Some Previous Observations on the Yolk Nucleus.*

It is not my purpose in the present paper to attempt a comprehensive review of the yolk nucleus.*

* For full historical accounts of the yolk nucleus, see JORDAN, 1893, p. 284; HENNEQUY, 1893, p. 20 (for Vertebrates); MERTENS, 1893, p. 389 (for birds and mammals); BAMBEKE, 1898, p. 571; and CRAMPTON, 1899.

I propose instead only to refer to those cases where the yolk nucleus, in its structural relations, seems to show a general resemblance to that of Antedon. Such yolk nuclei have been frequently described for the ova of widely separated animals; amongst those of recent years we may especially mention that described for Lumbricus by CALKINS (1895), for the diplopods Polyzonium and Blanijulus by NEMEC (1897), for the arachnid Pholcus by BAMBEKE (1898), and for the tunicate Molgula by CRAMPTON (1899). In spite, however, of the general, and indeed often striking, resemblance these and many other yolk nuclei show to that of Antedon, yet their mode of origin, fate, and general significance are made to differ widely from those I have described for the latter type. The suspicion that these differences may be due rather to the personal equation of the authors than to any such fundamental difference between the yolk nuclei themselves is considerably strengthened by the fact that, even for the yolk nucleus of Antedon itself, CRETY described a fate and assumed a general significance totally different from those which my own observations have led me to assume for this type. According to CRETY,* the yolk nucleus disintegrates, and the fragments so formed scatter over the egg and form fresh protoplasm, the yolk nucleus thus taking an active part in forming the plastic substances of the cytoplasm; a theory which is essentially in agreement with that originally propounded by SIEBALD in 1848, and subsequently adopted by numerous workers. A similar fragmentation of the yolk nucleus has been described by numerous recent authors, *e.g.*, MERTENS (1895), CALKINS (1895), NEMEC (1897), BAMBEKE (1898), and by CRAMPTON (1899).† These authors, however, interpret the fragmentation of the yolk nucleus as indicating an active participation of the latter structure in yolk formation. The yolk nucleus is thus assumed to take part in the formation of the metaplastic constituents of the cytoplasm. The products of disintegration are assumed to be either directly converted into the yolk spherules, *e.g.*, CRAMPTON (1898), CALKINS (1895), or indirectly causing or being utilised in their formation, *e.g.*, BAMBEKE (1898). As the latter author concludes for Pholcus (p. 537), the yolk nucleus is the formative centre for the yolk.‡ I have already shown that the appearances which led CRETY to assume that the yolk nucleus of Antedon undergoes a granular disintegration may be explained in quite another and far simpler manner, and I cannot help thinking that some similar explanation will be found to be true for other types also.

BAMBEKE'S account of the yolk nucleus of Pholcus is of special interest in connection with the present paper, for not only do the general form and changes in position of

* CRETY (*loc. cit.*), p. 266, "osservando ora la piccola macchia bruna rotondeggiante si scorge che da essa sembrano distaccarsi, come raggi, quei granuli minutissimi, che poi vanno a formare il discritto reticolo del plasma ovulare."

† For references to previous descriptions of yolk-nuclear disintegration, see BAMBEKE, 1893, pp. 537 and 547.

‡ MERTENS came to a similar conclusion for the yolk nucleus of vertebrates, and suggested the name "éléments vitellogènes" (1895, p. 417).

the yolk nucleus in this type strongly recall those shown by the yolk nucleus of Antedon, but this structure is in addition accompanied by a clear space in every way corresponding to that so often accompanying the yolk nucleus in Antedon. As in the latter type, this space usually lies on the outer, though sometimes also on the inner, side of the yolk nucleus. BAMBEKE concludes that this space represents an accumulation of fluid nutriment derived from without, and destined to be further elaborated by the yolk nucleus. It is difficult to imagine that appearances so closely similar as those presented by the yolk nucleus of Pholcus on the one hand, and of Antedon on the other, are correctly interpreted by such dissimilar conclusions as those of BAMBEKE for Pholcus and of myself for Antedon.

The egg of Molgula, as described by CRAMPTON in 1899, is another of the many cases in which a yolk nucleus, similar in general appearance to that of Antedon, has been described as disintegrating and taking an active share in yolk formation. CRAMPTON'S account of the yolk nucleus in Molgula is, however, of additional interest in that the mode of origin ascribed to this structure more closely resembles that which I have described for it in Antedon than does that as yet described for any other type. In Molgula the yolk nucleus arises as an ever-increasing accumulation in the cytoplasm of spherules identical in their staining reactions with certain spherules in the nucleus, and which by their acidophile staining reaction and disappearance when subjected to artificial gastric digestion appear to be albuminous in nature. The author concludes that the yolk nucleus in Molgula arises by the outward passage of these spherules into the cytoplasm from the nucleus, and regards this as another indication of the controlling influence exerted by the latter structure on the cytoplasm. The author does not, however, indicate the intranuclear source of these nuclear spherules, nor does he explain how they come to pass into the cytoplasm.

The acidophile staining reaction of the yolk nucleus in Molgula offers a striking contrast to the invariably intensely basophile reaction of this structure in Antedon; according to CRAMPTON, all basic stains, including HEIDENHAIN'S iron-alum-hæmatoxylin, cause the cytoplasm to be deeply stained while the yolk nucleus remains unstained. This reaction was obtained after the use of several fixing reagents, some of which, *e.g.*, saturated sublimate, I have also used in the present paper. The author notes that the basophile reaction of the cytoplasm, though strongly marked in the younger ova, slowly fades away as growth advances. A similar behaviour of the cytoplasm is described by MEAD (1897) for the ova of Chætopterus, and by GRIFFEN (1899) for those of Zirphæa. This resemblance in the staining reaction of the general cytoplasm to that which I have described for Antedon is considerably strengthened in the case of Molgula by the fact that in the latter type "sublimate fixation so affected the yolk as to make it retain the hæmatoxylin stain even after the chromatin became pale."

In striking contrast to the rational mode of origin suggested, though tentatively,

by CRAMPTON for the yolk nucleus of Molgula we have the accounts of MERTENS (1895) for various birds and mammals, CRETY (1895) for Distomum, HENNEQUY (1893) for Syngnathus, and BAMBEKE (1898) for Pholcus, by which the yolk nucleus is made to arise from a single small granule, which is ejected or budded off from the nucleus, and which subsequently attains its full size by independent growth in the cytoplasm, a mode of origin which, since it postulates a complex organisation and semi-autonomous metabolism for the yolk nucleus, is at least in harmony with the subsequent active share in yolk formation assumed by these authors for this structure.

XI. *Changes in the Cytoplasm (other than Yolk Nucleus) prior to Yolk Formation.*

In the young oocyte, previous to the formation of the definitive yolk nucleus, the cytoplasm is faintly acidophyle, showing little or no tendency to retain the basic stain. At about the time of the appearance of the definitive yolk nucleus, however, the cytoplasm commences to show a distinct basophile reaction, which steadily increases in intensity as the oocyte grows, until yolk formation commences. The basophile reaction of the cytoplasm, therefore, first becomes marked in eggs of from $30\,\mu$ to $40\,\mu$ in diameter, in which the nucleolus measures about $8\,\mu$, and in which the yolk nucleus is already deeply basophile. The reaction reaches its maximum in eggs of from $50\,\mu$ to $60\,\mu$ in diameter, in which the nucleolus measures $9\,\mu$ to $10\,\mu$, and gradually disappears as yolk formation progresses.

Unlike the yolk nucleus, the basophile reaction of which shows but little variation with different fixing reagents, the staining capacity of the cytoplasm varies greatly according to the fixing agent employed. The reaction is most marked in material fixed with a saturated solution of sublimate, the cytoplasm often being, with moderate differentiation, so deeply stained as to almost obscure the black yolk nucleus; greater differentiation will, however, always greatly de-stain the cytoplasm without appreciably affecting the appearance of the yolk nucleus.

After fixation with sublimate and acetic, the reaction of the cytoplasm is very similar, though slightly less intense, to that seen after sublimate fixation (fig. 34). In Hermann material, however, it is much weaker than with either of the above reagents, the eggs at the commencement of yolk formation staining a faint blue, but the yolk nucleus, though not so intensely stained as after sublimate fixation, is still deeply basophile (fig. 29). In material fixed with bichromate and acetic there is no indication whatever of a basophile staining reaction of the cytoplasm at the commencement of yolk formation, the eggs at this period actually staining more faintly than at any other time, but the yolk nucleus stains as intensely as in sublimate material (figs. 28 and 39). (Note that this material is also remarkable in that the formed yolk spherules are intensely basophile, instead of remaining, as after all other fixing reagents, practically unstained.)

The interest attaching to this variation in the staining capacity of the cytoplasm,

according to the nature of the fixing reagent employed, as emphasising the distinct nature of the causes determining this reaction on the part of the yolk nucleus and cytoplasm respectively, has already been noted (p. 468). This variation is also of interest in another connection. The progressively increasing staining capacity of the cytoplasm which precedes yolk formation may be assumed to be due to the gradual accumulation in the cytoplasm of material destined to be utilised later in the formation of the definite yolk spherules.* The variation in the staining capacity of the cytoplasm in the fixed material would depend on the capacity of the reagent employed to fix these metaplastic substances. They are, as we should expect, well fixed in sublimate material, and almost as well fixed in sublimate acetic material; in acetic bichromate material, on the other hand, they appear to remain wholly unfixed, and are washed out of the cell subsequent to fixation.

As we should expect, this varying action of the fixing reagent is accompanied not only by a corresponding variation in the staining capacity of the fixed cytoplasm, but also in its structural appearance. Thus in sublimate material, in which the basophile reaction is most strongly marked, the cytoplasm has the appearance of being composed of indefinite, closely packed, and in the earlier stages faintly staining, spherules. I have attempted to suggest this structure in fig. 21. In material fixed with an acid reagent, on the other hand, such, for example, as HERMANN's fluid, with which the basophile reaction is comparatively faintly marked, the texture of the cytoplasm is that of a more or less open, fine, and deeply-stained reticulum, the strands of which are, however, so fine that the cytoplasm as a whole does not appear deeply stained (fig. 17). Equally, of course, is this reticular structure of the cytoplasm found in material fixed with acetic bichromate, with which reagent, as already noted, the basophile reaction is almost, if not entirely, wanting (figs. 16 and 18). Such a behaviour of the cytoplasm would seem to suggest that the reticulum shown after fixation with an acid reagent represents the living protoplasm of the original cytoplasm, from which the more or less unfixed metaplastic substances have been washed out subsequent to fixation.†

Before leaving this description of the behaviour of the cytoplasm it should be noted that there is no trace, with any of the numerous fixing reagents I have employed, of the radiations out from the nucleus into the cytoplasm which have been described for this type by KORSCHELT (1889, p. 28, fig. 76), and for numerous other types by other authors.‡

* Note in this connection the frequency with which an increased staining capacity of the cytoplasm is described as accompanying the commencement of secretion in gland-cells.

† See WILSON's paper of 1899 on the Alveolar Structure of the Cytoplasm in the Ova of Echinoderms and other Animals.

‡ For other references to this and similar phenomena, see LUBOSCH's paper of 1902 (p. 774); also BAMBEKE, 1898 (p. 543).

XII. *The Formed Yolk.*

In the adult egg the yolk consists of closely crowded spherules of two kinds: the one kind, the yolk spherules proper, constituting the greater bulk of the cytoplasm, the other forming only a thin layer at the periphery of the egg, and which I will distinguish, for convenience of description, as the "peripheral spherules."

(a) *The Yolk Spherules: their Appearance with Varying Fixation.*—The yolk spherules proper average from 1–1·5 μ in diameter, and are always quite spherical in form. After fixation with sublimate or sublimate acetic, they remain unstained by the hæmatoxylin, and appear pale yellow in colour and refringent (fig. 65). After fixation with acetic bichromate, on the other hand, the yolk spherules are deeply basophile, prolonged extraction only serving to de-stain their extreme peripheral portions. After fixation with HERMANN's fluid, the spherules remain unstained, are non-refringent, and vary in colour from a pale yellow to a dark brown or even black. This coloration is due to the osmic acid in the fixing reagent,* and is entirely independent of staining or extraction, a fact which, together with their varying and irregular distribution, serves to sharply distinguish them from the peripheral spherules immediately to be described.

(b) *The Peripheral Spherules.*—These spherules, as already noted, form a thin layer immediately to the inside of the vitelline membrane. The surface of the adult egg, and with it the inner surface of the egg membrane, is, in fixed material, almost invariably thrown into fine wrinkles (fig. 65), the peripheral granules being collected into the ridges so produced. In the younger eggs this surface wrinkling is absent, and here the peripheral spherules form an even layer (fig. 63).

The peripheral spherules of the adult egg differ from those of the rest of the yolk both in their size and in their staining reactions. Their size, though averaging that of the yolk spherules, shows a considerably greater range of variation, spherules measuring as much as 3 μ across being occasionally met with. Their staining reaction, unlike that of the yolk spherules, shows no relation whatever to the mode of fixation; the spherules, after all reagents, being intensely stained with the iron hæmatoxylin. This staining reaction shows a yet further peculiarity, for in all cases very slight differentiation is sufficient to totally de-stain these spherules, and the ease with which this takes place is irrespective both of the nature of the fixing reagent and the duration of its action. In sublimate, or sublimate acetic material, with moderate extraction, the deeply stained peripheral spherules offer a striking contrast to the unstained yolk (fig. 65). With slightly greater extraction, insufficient, however, to appreciably affect the appearance of the other cell elements, the peripheral spherules are entirely de-stained, and they then appear as faintly yellow spherules of about the same size as the yolk spherules, but, unlike these, non-refringent. In

* HÄCKER, 1895, p. 219, describes a very similar appearance of the yolk after Hermann fixation in the ova of Canthocamptus.

acetic bichromate material the peripheral layer can still be detected, though the strongly basophile character of the yolk spherules after the use of this reagent renders it much less conspicuous. Here again, however, slight differentiation, to an extent which has no appreciable effect on the appearance of the yolk, suffices to totally de-stain the peripheral spherules, which then form a relatively clear region between the deeply stained yolk and the egg membrane. In Hermann material the peripheral spherules, when de-stained, cannot be distinguished from the faintly yellow and non-refringent yolk spherules; but with less differentiation they form with these as striking a contrast as in sublimate or sublimate acetic material. Occasionally, however, in the Hermann material, this contrast between the peripheral spherules and those of the rest of the yolk is more or less obscured by the arrangement of the more deeply coloured yolk spherules, to which reference has already been made (p. 479). The de-staining of the peripheral spherules is so sudden that, unless differentiation is carried out under the microscope, it is rare to obtain slides in which the peripheral layer is only partially de-stained. When, however, such a slide is obtained, it is seen that nearly all the still visible spherules are as large and as deeply stained as in less extracted slides, though present in far fewer numbers. Only here and there will a spherule have been caught in the act of de-staining, and then it is seen that, even in the case of the individual spherule, there is no gradual transition from an unstained to a deeply stained condition, for a part of the spherule still remains as deeply stained as before, while the rest—usually one side or the periphery, rarely the centre—is already completely de-stained. There is no evidence, in the de-staining of the peripheral layer during differentiation, that the more or less peripheral position of the spherules in any way influences the ease with which they part with the basic stain.

That the behaviour of the peripheral spherules is not due merely to their peripheral position is also indicated by the fact that in the adult egg they are never so closely crowded as to totally exclude the ordinary yolk spherules from this region, ordinary yolk spherules occurring among the peripheral spherules right up to the inner surface of the vitelline membrane.

It is, therefore, clear, from a study of the evidence afforded by the adult egg alone, that the peculiar behaviour of the peripheral layer of spherules is not of the nature of a fixation artifact, nor in any way due to the peripheral position which they occupy. It is, instead, evident that they must differ considerably in constitution from the yolk spherules. Nor is there any evidence that a conversion of the one into the other is taking place; a conclusion fully in harmony with the fact that the number of the peripheral spherules remains fairly constant, not only during the closing period of yolk formation, but also after the completion of this process for as long as the egg remains in the pinnule.

XIII. *Yolk Formation.*

(a) *Formation of the Yolk Spherules.*—The vacuolated areas indicating commencing yolk formation remain unstained after all fixing reagents, and in sublimate or

sublimate-acetic material they stand out in strong contrast to the intensely basophile cytoplasm. In acetic bichromate material the yolk spherules do not acquire their, with this reagent, intensely basophile character until some time after their first appearance. The best results are given by material fixed with HERMANN's fluid. With this reagent the vacuoles in the areas of commencing yolk formation can be seen on their very first appearance as faintly yellow spherules standing out well against the pale blue cytoplasm.

Isolated groups of yolk spherules, of a size equalling those of the adult egg, are frequently present long before yolk formation becomes general over the whole cytoplasm (figs. 29 and 34). These isolated areas of precocious yolk formation show, however, no constant spatial relation to any of the other cell elements, and as soon as yolk formation becomes general this process proceeds most rapidly at the cell-periphery, and as it extends from without inwards, the interspherular substance, and with it the basophile reaction of the cytoplasm, slowly disappear, the last traces forming a faint and irregular perinuclear area of ever decreasing dimensions (figs. 40 and 63).*

In addition to this complete absence of any spatial relation between the yolk nucleus and areas of commencing yolk formation, it should be noted that in the earlier stages of this process the yolk nucleus still retains its regular form, and shows as yet none of the appearances which have been previously interpreted as indications of its granular disintegration (figs. 29, 34, etc.). Moreover, in later stages yolk formation proceeds within the body of the yolk nucleus exactly as in other regions of the cytoplasm (figs. 38, 40 and 67).† The only difference which the yolk nucleus shows to the surrounding cytoplasm consists in the more intense staining reaction of the interspherular protoplasm in this region, a reaction which renders the yolk nucleus visible long after all tendency to retain the basic stain has disappeared from the surrounding cytoplasm. In the later stages of yolk formation the faintly stained and irregular yolk nucleus exactly resembles in appearance the still faintly basophile perinuclear region of the cytoplasm, and in many cases only its peripheral position serves to distinguish it from the latter region (fig. 40).

(b) *The Peripheral Spherules.*—As soon as yolk formation becomes general, the "peripheral spherules" make their appearance. As always, they are intensely basophile, but at this early stage they are extremely minute in size, far smaller than are the yolk spherules, and are scattered sparsely over the body of the cell (fig. 41). At a slightly later stage the spherules are more numerous, and show a considerable increase in size, though very small spherules are still abundant; and there is now a thin but distinct peripheral layer, formed mainly of the larger spherules, which lies

* MATHEWS (1899, p. 193), speaking of gland-cells generally, says: "The hylogens make their appearance in the gland-cell in a characteristic manner, almost invariably first appearing in the outer (lumen) end of the cell, farthest from the nucleus, and gradually approaching the latter."

† See also above, p. 473.

immediately on the inner side of the vitelline membrane (figs. 42 and 63). From this time onwards, as yolk formation progresses, the peripheral spherules continue to increase both in size and number, not only in the body of the cell, but also at the egg periphery (fig. 64).

As yolk formation approaches completion the spherules scattered over the cytoplasm decrease rapidly in number, though without showing any other change, and by the time the process is completed have almost or entirely disappeared from the body of the cell. At the same time, the peripheral layer increases considerably in depth and density, until, on the completion of yolk formation, it presents the appearance we have already described in the preceding section, and which is maintained for as long as the egg remains in the pinnule (fig. 65).

The behaviour of the "peripheral spherules" just described clearly indicates that they arise in the cytoplasm during yolk formation as a by-product of this process, and gradually accumulate at the surface of the cell. They have thus nothing to do with the discharged nucleolar spherules which—in the adult egg—they so frequently resemble, and their very different behaviour with fixing reagents, especially with sublimate solution, serves to distinguish them from the often contemporaneously appearing chromatin spherules. The entire absence of any spatial relation on the part of the peripheral spherules to the yolk nucleus, together with the behaviour already described for this structure, equally exclude the latter as a possible source for these spherules. The frequency with which spherules, often described as originating by the granular disintegration of the yolk nucleus, have been made to take an active part in determining yolk formation* necessitates considerable emphasis being laid on the mode of origin and fate of the "peripheral spherules" in Antedon.

XIV. *General Considerations on the Relation of Yolk Formation to the Cell-Activity.*

The process of yolk formation, that is the actual appearance of the definitive yolk spherules, occupies but a short time compared with the preceding period of growth. That this is so is shown, not only by the relatively slight increase in size of the nucleolus during this process, but also by the small number of ova in which yolk formation is actually taking place compared with those still preparing for this process.

I have shown that, in Antedon, during this period of yolk formation, there is not the slightest indication of increased activity on the part of the nucleus. I have also shown that material for the eventual production of the yolk spherules is gradually stored up in the cytoplasm throughout the whole of the preceding growth period of the egg.

The actual formation of the yolk spherules must therefore be regarded as an automatic process, which commences as soon as the accumulated materials in the

* *E.g.*, BAMBEKE, 1898, p. 550, for Pholcus.

cytoplasm attain the requisite degree of concentration, and which does not entail either increased nutrition of the ovum or increased activity of the nucleus.

Such an explanation is not only suggested by the suddenness with which yolk formation commences and the rapidity with which it is completed, but it is also in complete agreement with both the absence of correlation between the condition of the nucleus and the commencement of yolk formation described for so many eggs, and the variable position in the cell of the first formed yolk.

HÄCKER has come to the same conclusion on somewhat different grounds: he found that for the copepods Cyclops and Canthocamptus, not only was there no correlation between the phase reached by the germinal vesicle and the commencement of yolk formation, but the latter process was initiated by the passage of the egg into the oviduct; in the case of Canthocamptus, at the moment of reaching the proliferating portion of the wall of the mesenteron. HÄCKER suggested that there might be a real correlation between the nucleus and the "preparedness" of the cytoplasm for yolk formation, but that the latter condition did not become evident, as yolk formation, until the cytoplasm received the necessary stimulus.

It is probable, however, that the necessity for such a definite stimulus as that described for these copepods is exceptional, and that in the majority of cases, as I have suggested for Antedon, the mere concentration of the accumulated material in the cytoplasm suffices to start yolk formation.

The great majority of the papers dealing with this subject contain the implied suggestion that the whole process of yolk formation is embraced in the appearance of the yolk spherules. And for the majority of these authors, the nucleus, in addition to initiating, takes an active, and indeed controlling, share in the process. Setting aside those theories by which a yolk nucleus, itself of nuclear origin, is assumed to take an active part in yolk formation, we have the suggestion of MACALLUM (1894), based on the study of the ovarian eggs of Necturus and Rana, that "The peripheral nucleoli generate a substance, therefore, which diffuses gradually through the nucleus, then through the cell-protoplasm, the point of time of the latter occurrence corresponding with the formation of the yolk spherules." The suggestion thus implied, that the process of yolk formation commences only with the appearance of the yolk itself, accounts for the frequent endeavour to interpret changes presented by the nucleus during yolk formation as due to its active participation in the latter process. Thus BAMBEKE (1898, p. 564) interprets in this sense the changes in form of the nucleus, indistinctness of the nuclear membrane, and radiations into the cytoplasm from the latter, shown by the ovum of Pholcus at the commencement of yolk formation. The same assumption, that yolk formation commences only with the appearance of the yolk spherules, underlies LUBOSCH'S objection (1902) to BORN'S conception of the germinal vesicle stage as being one of greatly enhanced vegetative activity. LUBOSCH considers that the peripheral position of the first formed yolk, and the absence of any correlation between the commencement of yolk

formation and the condition of the nucleus, disprove BORN'S theory. Both these objections are removed by the conception of yolk formation which I have given above.

In this connection MUNSON'S account of Limulus is exceptionally interesting. In the ova of this animal, not only does yolk formation commence with the passage of the egg into the internal cavity of the ovary, but also with its separation from the follicle cells originally surrounding it. The egg is thus, at the commencement of yolk formation, cut off from the source which supplied it with food material during earlier stages, a fact which tends to confirm my suggestion, that "yolk formation" is only the final stage of a long period of metabolic activity.

There is yet another feature in the behaviour of the Limulus egg, as described by MUNSON, which tends to throw light on the question of the relation of yolk formation to the general cell-metabolism. It concerns the behaviour of the nucleolus. The separation of the egg from its follicle cells in Limulus is not the only event which synchronises with the commencement of yolk formation, for this moment is also marked by the total disappearance of the nucleolus. In considering the behaviour of the latter structure in Antedon, and in attempting to harmonise the absence of any trace of correlation between its activity and the commencement of yolk formation with the fact that this activity ceased entirely with the completion of the latter process, I suggested that the activity of the nucleolus might be concerned with a much more fundamental and wide-spread metabolic process of the cytoplasm than that which is represented by yolk formation, and that it is the cessation of this more fundamental process, at the completion of yolk formation, which brings about the cessation of nucleolar activity. I also pointed out that not only did such a suggestion remove the difficulty that the nucleolar activity was characteristic of the *whole* of the growth period of the egg preceding yolk formation, but that a large nucleolus is characteristic of many cells which differ widely in the result of their metabolic activity (*e.g.*, gland cells and ganglion cells). A comparison between the behaviour of the nucleolus in Antedon with that described for Limulus by MUNSON offers most striking confirmation of this suggestion that the nucleolar activity is related to the metabolism of the cytoplasm as a whole, rather than to the very small part of its activity which is represented by the conversion of the accumulated metaplastic material into a form sufficiently stable to survive the period of quiescence which succeeds the completion of the egg's growth. In Antedon, since there is no sudden change in the relation of the egg to its surroundings at the commencement of yolk formation, we may suppose that nutrition, and the accompanying processes of respiration and excretion, still continue, though doubtless in ever-diminishing intensity, and that they only finally cease with the cessation of all cell-activity which accompanies the completion of yolk formation. In Limulus, on the other hand, as the sudden break between the egg and its surroundings would lead us to expect, assimilation in the cytoplasm entirely ceases at the commencement of yolk formation; this cessation of assimilative activity on

the part of the cytoplasm being accompanied by a corresponding cessation (and in this case, total disappearance) of the nucleolus.

XV. *Significance of the Chromatin in the Growing Oocyte.*

I pointed out, when considering the significance of the nucleolus in Antedon (p. 461), that one of the reasons for excluding the raw food material dissolved in the caryolymph from the category of possible immediate sources for the nucleolar constituents was the strict correlation the size of this structure invariably shows to the phase of the egg's growth, a correlation very difficult to understand (in the absence of any indication of a reaction between the nucleolus and the caryolymph) if the growth of the nucleolus is due to the passive deposition on to its surface of substance derived directly from a source outside the cell. When, however, we come to consider the behaviour of the chromatin, we find that this structure, unlike the nucleolus, does show a very obvious endeavour, which is indicated by its subdivision and equal distribution, to increase the facilities for reaction with the surrounding caryolymph. This behaviour must indicate, not merely that the chromatin depends, for the material on which it works, on substances dissolved in the caryolymph, for so does the nucleolus, but that these substances are not deposited, as such, on the chromatin, but have instead to undergo their initial changes under the direct influence of this cell-structure while they are still dissolved in the caryolymph. In other words, the chromatin obtains its food material by active incorporation, whereas the nucleolus grows by passive deposition. The obvious source for the food material of the chromatin, which thus, unlike that of the nucleolus, shows so little relation to the requirements of particular cell-structures, is the nutritive and oxygenated plasma of the parent organism which enters the tissues of the ovary from the surrounding sinus.*

We have already seen reason for assuming the passage of substance from the chromatin to the nucleolus; there is also evidence for the passage of substance from the chromatin to the cytoplasm. During the early growth of the oocyte, prior to yolk formation, the cytoplasm is not only increasing in quantity, but is also steadily accumulating material to be subsequently utilised in yolk formation, this accumulation being indicated by the progressively increasing basophile reaction of the cytoplasm (see also p. 477). During this period the chromatin, from being massed round the nucleolus, has become finely subdivided, and has, at the same time, lost much of its affinity for the basic stain.† With the commencement of yolk formation, on the other hand, the chromatin shows a progressively increasing staining capacity, and at the same time deeply staining droplets appear on the

* Note in this connection the invariably superficial position in the ovary of the growing oocyte at a time when this activity of the chromatin is at its maximum. (See also p. 450.)

† *Cf.* fainter staining of nucleus and deeper staining of cytoplasm in gland-cells at commencement of secretion. *E.g.*, LAUNAY, 1902 (cells of parotid gland).

threads and gradually increase in number and size. I interpret the faintly stained condition of the chromatin prior to the commencement of yolk formation as due to the avidity with which the cytoplasm at this stage takes up the products of the chromatin activity. The caryolymph is thus always unsaturated and absorbs this material as fast as the chromatin can produce it. With the commencement of the—probably automatic—changes in the cytoplasm by which this accumulated material is converted into the definitive yolk spherules, there is, as we should expect, a marked diminution in the avidity with which the cytoplasm absorbs the products of chromatin activity. The chromatin, however, continues, for a time at least, to produce them in as great a quantity as before, but the caryolymph, being now saturated, no longer removes them, and they therefore slowly accumulate on the chromatin threads, causing both the deeper staining of the latter and the formation of the chromatin spherules.

XVI. *Relative Sizes of the Egg, Nucleus and Nucleolus.*

Up to the commencement of yolk formation the nucleus, like the nucleolus, increases steadily in size step by step with the growth of the cytoplasm, the ratio of their diameters remaining almost, if not quite, constant. Thus the nucleus is usually a little over a half, the nucleolus a little under a quarter, the diameter of the egg. The following measurements show this relation at different periods of the egg's growth prior to yolk formation, the figure given for each stage being the mean of a considerable number of closely approximating measurements.

Nucleolus.	Nucleus.	Cell
μ.	μ.	μ.
2·5	6·5	10
3·5	10	18
5	14	20
7	18	30
8	20	30–40
9·5	25	45

With the commencement of yolk formation, however, this correlation in growth ceases, and the cell body grows, or, to speak more correctly, expands, at a much quicker rate than do the other cell structures; so that on the completion of yolk formation the nucleolus measures about 17 μ, the nucleus 50 μ, while the cell body measures fully 160 μ. The following figures represent typical measurements during the progress of yolk formation; they were obtained in the same way as those just given for earlier stages, the mean being taken of a large number of closely approximating measurements for each stage. It should be noted, however, in considering these figures, that although the nucleolus continues to increase in size perfectly steadily during yolk formation, a corresponding behaviour could not

satisfactorily be determined for the nucleus, the irregular form of the latter at this period quite precluding accurate measurement. The figures for the nucleus, therefore, in the following table are only approximately correct.

Nucleolus.	Nucleus.	Cell.
μ.	μ.	μ.
12	35–40	50–80
15	40	135
17	50	160

LUDWIG, 1877 (p. 288), says for Antedon Eschrichti : "lässt sich durch Messungen leicht feststellen, dass Dotter, Keimbläschen und Keimfleck in ihrer Grössenzunahme nicht ganz gleich Schritt mit einander halten." His measurements, however, though greater than mine, corresponding with the larger size of the egg in this species, show exactly the same relative proportions for the nucleolus, nucleus and cell body. That is to say, the nucleolus is a little under a quarter, the nucleus a little over a half, the size of the egg, this ratio only disappearing at the commencement of yolk formation. JORDAN, in his account of the Newt (1893), gives a very similar relation between the germinal vesicle and the egg to that described in the present paper for Antedon, the germinal vesicle being half the size of the egg until the commencement of yolk formation.

XVII. *The Form of the Nucleus.*

One of the most striking features of the young oocyte is the extreme regularity of its spherical nucleus. With no fixing reagent have I ever found this structure in early stages show the slightest tendency to assume an irregular form. In striking contrast to this behaviour of the nucleus in the younger oocytes, this structure in later stages is almost always more or less irregular in form, and in some material is markedly so (figs. 30, 32, 33, 40). This irregularity coincides with the period during which the cytoplasm is deeply basophile, becoming more and more pronounced the deeper the cytoplasm stains, and gradually passing away as this staining capacity of the cytoplasm is lost during yolk formation, the nucleus never, however, regaining the regular spherical form characteristic of the earlier stages. The irregularity of the nucleus thus coincides, not with the actual process of yolk formation, but with the basophile staining reaction of the cytoplasm.

There are three main views as to the significance of this nuclear irregularity. Either it is regarded as due to spontaneous amœboid movements on the part of the nucleus itself, and interpreted as indicating a direct participation of this structure in yolk formation (KORSCHELT, BAMBEKE, etc.),* or as due to the pressure exerted by the food-laden cytoplasm, the nucleus playing a purely passive part (NUSSBAUM), or

* For full discussion and review of nuclear irregularity, see KORSCHELT, 1898, and BAMBEKE, 1898 (p. 556). KORSCHELT interprets in this sense the nuclear irregularity in Antedon itself.

finally, the irregularity is regarded as an artifact—a result of the action of the fixing reagent.

There are several facts which show that, for Antedon, the last explanation is the correct one. In the first place the nuclear irregularity shows no spatial relation whatever, either to the other cell-structures, to commencing yolk formation, or to the position of the nucleus in the cell. In the second place, it is only in radial section that the nuclear irregularity presents the appearance of Pseudopodia; in tangential sections these nuclear "processes" are found to invariably resolve themselves into a coarse wrinkling of the nuclear membrane (figs. 68 and 69). Finally, the artificial nature of the nuclear irregularity is strongly indicated by the variable behaviour of the nucleus with varying fixation. Only in material fixed with sublimate is the irregularity of the nucleus invariably well marked. With other reagents, *e.g.*, sublimate acetic, bichromate acetic, or HERMANN'S fluid, the irregularity may be much less marked or even entirely absent, and this is true even of material taken from one and the same individual.

Although the nuclear irregularity is thus seen to be dependent on the action of the fixing reagent, yet the striking temporal relation which this irregularity shows to the condition of the cytoplasm, and thus to the egg's growth, indicates that its occurrence is also dependent, in part at least, on a physiological condition of the egg peculiar to this period. The fact that the nuclear irregularity is always well marked in material fixed in sublimate solution alone, while it is much less marked, or even entirely absent, when the fixing reagent employed contains a free acid, would seem at first sight to suggest that the nuclear irregularity may be due to the slow action or penetration of the fixing reagent.* This suggestion, however, entirely fails to explain the cause of the direct temporal relation between the nuclear irregularity and the stage of the egg's growth, as indicated by the condition of the cytoplasm. For not only is the egg, at the stage marked by the greatest nuclear irregularity and cytoplasmic staining capacity, invariably at the surface of the ovary, and therefore in a position to be most quickly reached by the fixing reagent, but the younger eggs, in which the nucleus is perfectly spherical, are usually in the interior of the ovary, either floating freely, or tightly packed between the larger eggs. Moreover, eggs at this early stage are occasionally found at the surface of the ovary, yet their nuclei are no less regularly spherical. In the same way the form of the nucleus in adult, or nearly adult, eggs shows no relation whatever to the position of the egg relative to the surface of the ovary. The position of the egg in the pinnule, and thus also the facility offered for rapid penetration by the fixing reagent, cannot, therefore, be a factor in determining the irregularity of the nucleus.

We come now to the consideration of the cytoplasm, the only cell constituent to the condition of which the nuclear irregularity shows any definite relation, and here, I believe, we find the true cause of the nuclear irregularity seen in fixed material.

* See p. 490, *re* similar suggestion by JORDAN.

In the younger oocytes the spherical form of the nucleus is determined by the osmotic interchange taking place between the nutritive plasma of the ovary on the one hand, and the caryolymph within the nucleus of the oocyte on the other. As the egg grows there is a gradual accumulation of metoplastic material in the cytoplasm, the presence of which must very greatly increase the osmotic intensity at the cell wall while producing a corresponding decrease in the osmotic intensity at the nuclear membrane. The nucleus, therefore, no longer possesses the same powerful tendency to retain a spherical shape, and although it is possible, indeed probable, that the actual deviations from this spherical form in life are slight, they may obviously be greatly intensified by the action of the fixing reagent in coagulating the cytoplasm.

As yolk formation progresses the cytoplasm gradually loses its fluid character, for although the yolk spherules are themselves as fluid as the original food-laden cytoplasm (*cf.* WILSON, 1899), yet the conversion from a fine to a coarse alveolar structure is necessarily accompanied by a firmer consistency of the alveolar mass as a whole. The nucleus, therefore, is no longer free to assume a markedly irregular form during fixation, or, to put it another way, the firmer consistency of the coarsely alveolar cytoplasm results in less movement of the latter during fixation, and therefore less disturbance in the form of the nucleus.

XVIII. *Some previous Observations on the Form of the Nucleus.*

As KORSCHELT remarks in his paper of 1889, a departure from the usual regular form is one of the most evident changes undergone by the nucleus. Irregularity of the nucleus was, therefore, early noted, and has been described for the cells of various tissues, and especially for actively secreting gland cells, the nuclei of these latter cells often forming a branched system extending throughout the cell.* In the case of the ovum, the irregularity is usually much less marked, and appearances are more suggestive of amœboid movements—a suggestion considerably strengthened by the slow changes in form described for the living ovum by VOGT in 1878 and by many later authors. KORSCHELT, in the paper just referred to, interprets this irregular form of the nucleus, in both glands, cells and ova, as being due to the necessity for increased contact surface between the nucleus and the cytoplasm. In support of this suggestion, the author not only quotes from the literature to show that the irregularity of the nucleus in gland cells varies with the cell's activity,† but he also describes for the ovum of Dytiscus a direct relation between the nuclear irregularity and local accumulations of food material in the cytoplasm. A similar spatial relation between the nuclear "Pseudopodia" and local conditions in the cytoplasm is described

* See KORSCHELT, 1889, for numerous figures of branched nuclei of both nutritive and gland cells. Compare the richly chromatic character of these nuclei with the achromatic condition of the, for the most part, spherical germinal vesicles shown on the same plates.

† *E.g.*, HEIDENHAIN, 1883, "Parotid of Rabbit"; see also SCHMIDT, 1882.

by BAMBEKE (1898) for the ovarian egg of Pholcus. This author also comes to the conclusion that the irregularity of the nucleus is not an artifact, but is due to the active participation of the nucleus in the cell-metabolism, and results from an endeavour on the part of the latter to increase the opportunities for reaction at its surface with the surrounding cytoplasm. Both these authors, like many others, describe a partial disappearance of the nuclear membrane, and accept this as a further indication of the controlling influence exerted by the nucleus on the cell-metabolism.

The suspicion that, in these spontaneous movements of the nucleus on the one hand, and the fixation artifacts I have described for Antedon on the other, we have to deal with identical phenomena is considerably strengthened by the fact that, as we have seen was the case in the yolk nucleus, these conflicting accounts have also been given for the nucleus of Antedon itself. Thus KORSCHELT* figures and briefly describes the nuclear irregularity in the ova of Antedon, and interprets it in the same way as for other types.

Spontaneous amœboid movements on the part of the nucleus, such as BAMBEKE KORSCHELT, and others have assumed, is not the only explanation that has been put forward. NUSSBAUM (1882), as also CONKLIN (1897), suggested that these movements might be rather due to an invasion or pressure on the part of the cytoplasm than to active movements on the part of the nucleus itself. JORDAN, in his paper of 1893 (Newt) describes that, though he has found slight nuclear irregularity with other fixing reagents, he has never once seen it after fixation in hot water. He therefore suggests that nuclear irregularity, in some cases at least, may be due to the slow approach of the fixing reagent. JORDAN, however, regards this irregularity as due to the "irritation" of the slowly approaching reagent, and therefore as indicating a potential capacity on the part of the nucleus for spontaneous amœboid movement which is normally latent in the Newt, and he agrees with KORSCHELT that these movements in other types probably have a nutritive significance. In striking contrast to JORDAN'S suggestion, KORSCHELT, in 1898, suggested that the irregular form of the nucleus in fixed material might be due to *rapid* fixation, slow fixation allowing the nucleus to return to a spherical condition.

XIX. *The Nuclear Membrane.*

The existence of a distinct nuclear membrane has been frequently accepted as incompatible with the discharge of spherules as such from the nucleus into the cytoplasm, and the partial or complete disappearance of this membrane has been interpreted as indicating an increased reaction between the nucleus and the cytoplasm. As my conclusions for Antedon assume the discharge of formed spherules into the cytoplasm, though a distinct nuclear membrane be present, and as my interpretation of the cell processes takes no note of the condition of the nuclear membrane, it is necessary to offer some explanation of the latter structure which shall not necessitate

* KORSCHELT, 1889, p. 27, and figs. 74 and 75.

the assumption that the existence of a clearly defined nuclear membrane in any way interferes with an active reaction between the nucleus and the cytoplasm, or that it possesses in the living egg the firm consistency which it often appears to possess in fixed material. I believe that as a result of my observations on Antedon I am able to offer an explanation which will fulfil these conditions.

The nuclear membrane is always present in the oocyte of Antedon.* Only when the nucleus is very irregular in form does the nuclear membrane appear to be interrupted. The partial disappearance of the limiting membrane in these nuclei is, however, always directly associated with the irregularities in the nuclear outline, and is, I believe, due to the nuclear membrane of these points being cut in a very oblique direction—a natural result of the wrinkled form of the nucleus. This suggestion receives considerable support from the appearance of tangential sections of the nucleus, in which it can be readily seen that the nuclear membrane is perfectly well marked on any part of the nuclear outline which does not quickly change its position in neighbouring sections of the series, but is invisible in those regions of the outline which do quickly alter their position in adjacent sections, and which thus show that they are cut extremely obliquely. The frequency with which this local disappearance of the nuclear membrane has been described as associated with nuclear irregularity suggests that the above explanation will be found to apply to these other cases also.†

Although we may, therefore, assume that the nuclear membrane is always present at all stages of the oocyte's growth, it nevertheless varies greatly in appearance according to the nature of the fixing reagent employed. It is, for example, always difficult to see after the use of sublimate, while in Hermann material, and indeed after the use of all reagents containing a free acid, it is not only quite distinct, but usually deeply stained. It is thus seen that the behaviour of the nuclear membrane shows a striking parallelism with that already described for the cytoplasm (see p. 478). Those reagents which give a well fixed cytoplasmic reticulum give also a distinct nuclear membrane, while those reagents, such as sublimate, after the use of which the cytoplasmic reticulum is absent or masked by the metaplastic substances, give a nuclear membrane which is indistinct and often difficult to detect. When there is a distinct cytoplasmic reticulum, as for instance, after the use of HERMANN'S fluid, the nuclear membrane appears to be continuous with the strands of the latter. I cannot but think that this behaviour indicates that the nuclear membrane consists of a layer of the living protoplasm, free from the metaplastic substances with which the rest of the cytoplasm is loaded. The formation of such a layer of "hyaline" protoplasm at the junction of the cytoplasm with the caryolymph is to be explained as due to physical causes, and is comparable with the hyaline layer of amœba.

* LUDWIG (1877, p. 289) says that the germinal vesicle possesses a "deutlich doppelcontourirte Wandung." I have never seen anything to suggest this appearance.

† For references to observations on this subject, see BAMBEKE, 1898, p. 544.

Such a conception of the nuclear membrane not only allows its formation to be readily explained as due to physical causes, but also offers a ready explanation of the ease with which this structure can be absorbed and reformed at different periods in many cells. Moreover, it would certainly seem that the living protoplasm of the cell is the most natural material of which the membrane, through which all solutions must diffuse before entering the nucleus, would be composed.

XX. *Suggestions as to the Nature of the Factors Determining the Arrangement of the Chromatin and Form of the Nucleus in the Growing Oocyte.*

There are two very characteristic features in which the germinal vesicle differs from the nucleus of actively secreting somatic cells: these are (*a*) the relative paucity and fine subdivision of its chromatin, and (*b*) its large size and (usually at all events) spherical form.

I believe I am able to offer an explanation of both these features of the germinal vesicle which will be in complete harmony with the interpretations and conclusions I have been led to form as a result of the study of the oocyte of Antedon, and which will, moreover, bring the behaviour of the latter into line with that, not only of somatic gland cells, but also of abortive ova functioning as nurse cells.

(a) *The Paucity and Fine Subdivision of the Chromatin in the Germinal Vesicle.*—It is generally assumed that the chromatin of the germ cell constitutes the main, if not the only, carrier of the specific characters. If this assumption be accepted, then it is not unreasonable to suppose that this consideration must place a very considerable limitation on the adaptive changes which the chromatin may undergo during the increase of the cell-activity which accompanies the processes of growth and yolk secretion. The somatic gland cell, on the other hand, as also the abortive egg or nurse cell, are not destined to give rise to a long line of descendants; still less are they responsible for the unimpaired preservation of the specific characters of the race. Their chromatin, therefore, is free from the limitations which I have assumed to exist in the case of the oocyte, and is thus capable of very considerable adaptation to the requirements of the cell's activity. We thus find that, while the nucleus of the gland cell and nurse cell speedily becomes richly chromatic as the cell-activity increases, that of the egg remains relatively poor in chromatin.

If such a limitation to the growth of the chromatin in the oocyte does exist, then we have a ready explanation of the fine subdivision and equal distribution of this material so frequently found in the germinal vesicle which will permit of our following RÜCKERT* in interpreting this condition as correlated with intense nuclear activity, although it is absent in the equally active nuclei of gland and nurse cells. For it is obvious that this limitation will necessitate what chromatin there is being used

* J. RÜCKERT, 1892, "Zur Entwicklungsgeschichte des Ovarialeies bei Selachiern" ('Anat. Anz.,' vol. 7, p. 127).

to the greatest possible advantage, and no clearer indication of such an economical utilisation of the chromatin could be offered than a fine subdivision and equal distribution of this material.

(b) *The Large Size and Spherical Form of the Germinal Vesicle.*—I have suggested (p. 489) that the spherical form of the germinal vesicle is due to the latter constituting an active osmotic system, and that the occasional deviation from this form which is seen in fixed material is an artifact, due to the action of the fixing reagent at a time when the osmotic intensity has been considerably lessened by changes in the cytoplasm. The large size of the germinal vesicle is to be explained as due to the same cause as that which brought about the fine subdivision of the chromatin, namely, the limited growth allowed to this material in the germ-cell. For it is obvious that there are two ways of enabling a small amount of chromatin to readily perform the utmost possible amount of work. One of these is the great increase in surface area which is brought about by extreme subdivision, whereby the facilities for reaction with the caryolymph are greatly augmented. The other is the increase, both in quanity and in nutritive value, of the caryolymph itself, which is brought about by an increase in the surface area of the nuclear membrane through which diffusion takes place, the size of the germinal vesicle being determined when equilibrium is established between the rate of diffusion of nutritive substances through the nuclear membrane on the one hand, and the requirements of the chromatin on the other.*

Summary.

The Yolk Nucleus.—Throughout the growth of the oocyte the nucleolus intermittently discharges groups of deeply basophile spherules into the cytoplasm. In the young oocyte these nuclear spherules remain unchanged, slowly accumulating in the cytoplasm, where they form small groups near the germinal vesicle. In slightly older oocytes the increased fluidity of the cytoplasm which results from the progressive accumulation of metaplastic material in preparation for yolk formation causes the discharged nucleolar substance to lose its spherular form and to diffuse on to the surrounding cytoplasm. The more deeply staining area of the cytoplasm to which this diffusion gives rise is the yolk nucleus. As the cytoplasm becomes still more fluid the yolk nucleus assumes the regular form of a concavo-convex lens embracing the spherical germinal vesicle, and thus behaves as a fluid mass under the influence of capillary forces. During yolk formation the yolk nucleus passes to the periphery of the egg, a migration also shared by the "peripheral spherules." The actual increase in size which the peripherally placed yolk nucleus shows during the earlier stages of yolk formation is speedily masked by the great

* Boveri (1904, p. 17), as a result of experiments on the development of nucleated and enucleated sea-urchin egg fragments, points out that it is the surface area of the nucleus, and not the nuclear volume, which is proportional to the chromatin contents of the nucleus.

superficial extension and thinning out on the surface of the egg which this structure undergoes during yolk formation, and which is the necessary result of the great increase in bulk and surface area which accompanies this process. As yolk formation progresses the yolk nucleus gradually loses in staining capacity, and before this process is completed has entirely disappeared from view. At no time, however, does the yolk nucleus show any indication of a granular disintegration, nor does it show any spatial relation to yolk formation, the latter process taking place in this area of the cytoplasm exactly as elsewhere. The clear area accompanying the yolk nucleus, usually on its outer surface, is an artifact and results from the inability of the acid fixing reagent to coagulate the metaplastic substances with which the cytoplasm, and especially the yolk nuclear area of the cytoplasm, is loaded. With saturated sublimate no such space is formed.

The yolk nucleus, therefore, is simply a region of the cytoplasm on to which material discharged from the nucleolus has diffused. The period at which this diffusion occurs, as also all subsequent changes in the form and position of the yolk nucleus, are determined by the progressive change in the physical consistency of the cytoplasm which precedes and accompanies yolk formation. The yolk nucleus, therefore, like the waste material discharged from the nucleolus subsequent to its formation, takes no part in the cell processes.

The Nucleolus.—The spherical form of the nucleolus, a form in which minimal surface area is associated with maximal bulk, stands in striking contrast to the subdivided condition of the chromatin, and indicates the absence of any reaction between the caryolymph and the nucleolar substance at the surface of the nucleolus, the nucleolus growing by the passive deposition of material on its surface from solution in the caryolymph.

This nucleolar material consists of two substances: the one acidophile and extending throughout the nucleolus, the other deeply basophile and borne by the acidophile ground substance, to which its presence imparts a considerably firmer consistency.

Nucleolar activity, which proceeds from within outwards, results in the breakdown of the basophile constituent, the products of this process accumulating as an irregular zone between the firm, as yet unaltered, basophile cortical region and the acidophile internal region of the nucleolus. When the internal pressure reaches a certain point, the cortical layer is ruptured and the accumulated products are discharged into the caryolymph, partly as deeply basophile spherules, partly as a clear fluid. The spherular form of the discharged material is determined by the fluid character of the surrounding caryolymph, and when the accumulated products of nucleolar activity happen to lie within a vacuole, then they here also possess a spherular form. There is no indication that the acidophile ground substance of the nucleolus is itself discharged.

During the growth of the oocyte the amount of the deeply basophile nucleolar

substance varies greatly, but the growth of the nucleolus nevertheless shows a strict correlation with that of the egg, and is due to the steady accumulation of the acidophile ground substance.

On the completion of yolk formation all indications of nucleolar activity abruptly cease. The supply of the basophile constituent of the nucleolus also ceases, but the acidophile constituent continues to be deposited on to the nucleolar surface where, no longer being incorporated by the now inactive nucleolus, it forms lens-shaped accumulations.

The selective action shown by the nucleolar material, together with the strict correlation the growth of this structure shows to that of the egg, excludes the possibility of this substance being derived directly from a source outside the cell. The fact that the ground substance continues to be deposited on to the nucleolus after the completion of all cytoplasmic activity, together with the close relation this structure shows to the chromatin in earlier stages, points to the latter as its source, while the failure at the same time of the supply of the basophile constituent indicates the origin of the latter from the cytoplasm.

The steady growth of the nucleolus is not due to the accumulation of waste material, but to an increased production of the "ground substance" by the chromatin in order to cope with the ever-increasing production of waste material by the cytoplasm of the growing egg. From the nucleolus this waste material, now presumably inert and harmless, is discharged into the cytoplasm, where it slowly dissolves away.

The Chromatin.—With the increase in metabolism which accompanies the growth of the oocyte there is a corresponding finer subdivision and more uniform distribution of the chromatin. This endeavour to increase the facilities for reaction with the surrounding caryolymph indicates that the chromatin obtains its food material by active incorporation instead of by passive deposition. The absence of any reciprocal action on the part of the food material of the chromatin indicates for this substance a source outside the cell.

The elaborated food material is passed on from the chromatin in part to the nucleolus, but mainly to the cytoplasm. During the period preceding yolk formation, which is marked by the progressive accumulation of metaplastic material in preparation for this process, the avidity with which the cytoplasm takes up the products of chromatin activity causes the latter structure to appear faintly stained. With the commencement of yolk formation this avidity is greatly reduced, and the products of the chromatin activity, now being produced in excess of the requirements of the cytoplasm, accumulate on the chromatin threads, causing the increase in the staining capacity of the latter and the formation of the basophile droplets.

The Cytoplasm.—Throughout the whole growth period of the oocyte there is a gradual accumulation in the cytoplasm of metaplastic material in preparation for yolk formation. This accumulation is accompanied by an increase in the basophile

staining capacity, and fluid consistency, of the cytoplasm. The process of yolk formation, that is, the actual appearance of the definitive yolk spherules, is unaccompanied by increased nuclear or nucleolar activity, and consists simply in the rapid and automatic conversion of the accumulated material into a form sufficiently stable to survive the period of quiescence which succeeds the completion of the egg's growth.

The "Germinal Vesicle."—Form of the nucleus. The oocyte constitutes an osmotic system, of which the cell wall forms an outer, the nuclear membrane an inner, semi-permeable membrane. The accumulation in the cytoplasm of soluble substances in preparation for yolk formation causes an increase in the osmotic intensity at the outer membrane, and a corresponding decrease of that at the inner. The loss of turgescence which thus results permits of the assumption of an irregular form during fixation.

Size of the nucleus and subdivision of the chromatin. The large size of the germinal vesicle, as also the fine subdivision of the chromatin, are due to the limitations which the care of the specific qualities imposes upon the adaptive capacity of the chromatin; both these conditions being merely the expression of a more economic use of this material. The subdivision of the chromatin increases the area of contact with the caryolymph, and the increased area of the nuclear membrane through which diffusion takes place permits of a greater concentration of nutritive material within the nucleus. Up to the commencement of the expansion due to the formation of the definitive yolk spherules, therefore, the size of the germinal vesicle shows a close relation to that of the egg, and is determined when equilibrium is established between the rate of diffusion of nutritive substances through the nuclear membrane on the one hand and the requirements of the chromatin on the other.

EXPLANATION OF PLATES 29-31.

All the figures were drawn under a Zeiss 2 mm. apochromatic objective, in the great majority of cases with the aid of the camera. Some of the nuclei in Plate 29, and some of the nucleoli in Plate 30 were, however, drawn by direct measurement with the ocular micrometer. In all cases the drawings were checked by direct measurement.

Plate 29.

Fig. 1.—Resting and freely floating oogonia from "BNO" material. The faintly stained nucleolus stands in sharp contrast to the enclosing chromatin. × 2000.

Fig. 2.—Double nuclei from a quite immature ovary. × 2000.

Fig. 3.—Early stage in synapsis, the split and segmented spireme thread is aggregating round the spherical and sharply-marked nucleolus. × 2000.

Fig. 4.—Synapsis stage; the longitudinal split is still distinctly visible. × 2000.

Fig. 5.—Synapsis stage; the longitudinal split is only doubtfully visible. × 2000.

Fig. 6.—Resolution of the synapsis; the nucleolus is again visible and perfectly normal in appearance. × 2000.

Figs. 7 and 8.—Formation of the definitive U-shaped chromosomes; the latter lie on the nuclear membrane. The nucleolus is central in position and still normal. In fig. 8 two of the chromosomes are still longitudinally split. × 2000.

Fig. 9.—Aggregation of the definitive chromosomes in the centre of the nucleus; the nucleolus is not visible at this stage. × 2000.

Fig. 10.—The completed mitotic figure. At this stage also there is no trace of the nucleolus. × 2000.

Fig. 11.—Two oocytes shortly after the completion of the oogonial mitosis. (Nucleus = $4 \cdot 5\ \mu$; nucleolus = $1 \cdot 25\ \mu$.) There is only a faint indication of a nuclear reticulum. An oogonial nucleus in prophase for mitosis is shown in close association. × 2000.

Fig. 12.—A somewhat older oocyte. (Nucleus = $7\ \mu$; nucleolus = $2 \cdot 5\ \mu$.) The linin reticulum is distinctly visible, and the chromatin is commencing to extend out from the surface of the nucleolus. × 2000.

Fig. 13.—A similar stage in a slightly older oocyte. (Nucleolus = $3 \cdot 5\ \mu$.) The nucleolus shows an excentrically-placed de-stained area, and a group of "nucleolar" spherules is already present in the cytoplasm. × 2000.

Fig. 14.—Oocyte in which the chromatin extends as radial strands from the nucleolus to the nuclear membrane. (Nucleus = $7\ \mu$; nucleolus = $2 \cdot 5\ \mu$.) × 2000.

Fig. 15.—Somewhat older oocyte than that shown in fig. 14. (Nucleus = $10\,\mu$; nucleolus = $3{\cdot}5\,\mu$.) The (in this material) faintly stained chromatin is still radially arranged. Several groups of "nucleolar" spherules are present in the cytoplasm, and four spherules in the nucleus show a suggestive spatial relation to those in the cytoplasm. This figure shows well the spherical form, sharp outline, and variable size, of these nucleolar spherules. × 2000.

Fig. 16.—Slightly older oocyte, from bichromate-acetic material. (Nucleolus = $5\,\mu$.) The chromatin is on the point of losing its connection, both with the surface of the nucleolus and with the nuclear membrane, and the strands are now distinctly granular. × 2000.

Fig. 17.—Oocyte from Hermann material. (Nucleolus = $3{\cdot}5\,\mu$.) Although the slide from which this section was taken was deeply stained, yet the nucleolus is faintly stained and stands in sharp contrast to the chromatin (*cf.* also fig. 54). Several groups of nucleolar spherules are present in the cytoplasm. × 2000.

Fig. 18.—Oocyte from bichromate-acetic material. (Nucleolus = $6\,\mu$.) The chromatin is now in the form of scattered branched threads. The deeply stained and excentrically-placed nucleolus is seen in the act of discharging a stream of nucleolar spherules into the cytoplasm. A group of similar spherules is seen to the left. × 2000.

Figs. 19 and 20.—Oocytes showing double nucleolus. (Nucleolus = $3{\cdot}5\,\mu$ and $2{\cdot}5\,\mu$: $2{\cdot}5\,\mu$ and $5\,\mu$). This condition is rarely met with at later stages. There are several groups of nucleolar spherules in the cytoplasm and a few in the nucleus. The chromatin is still radially arranged, and is (as in all but the older eggs of this particular material) faintly stained. (*Cf.* also figs. 22, 23, 38, and 55, all taken from the same material.) × 1000.

Fig. 21.—Oocyte from sublimate material. (Nucleolus = $6\,\mu$.) The numerous nucleolar spherules in the cytoplasm are undergoing diffusion. × 1000.

Fig. 22.—Two adjacent oocytes. (Nucleoli = $3\,\mu$ and $7\,\mu$.) In the older the diffusion of the nucleolar spherules is in progress, and the more recently discharged spherules offer a striking contrast to the more deeply stained cytoplasmic area to which this diffusion gives rise. (*Cf.* also previous figure.) The younger oocyte shows a group of nucleolar spherules in the cytoplasm which are almost as large as those seen in the larger oocyte. (*Cf.* also figs. 19, 15, etc.) × 1000.

Fig. 23.—Oocyte at a slightly later stage than the larger one in fig. 22. (Nucleolus = $8\,\mu$.) The more deeply staining area of the cytoplasm is concentrating to form the definitive yolk nucleus, and there are also present three groups of as yet unaltered spherules. × 1000.

Fig. 24.—A similar oocyte (nucleolus = $8\,\mu$), but in which the nucleolus is seen in the

act of discharging a group of nucleolar spherules from the de-stained area. As in the preceding figure, several nucleolar spherules are seen in contact with the forming yolk nucleus. $\times$ 1000.

Fig. 25.—An oocyte from sublimate-acetic material. (Nucleolus $= 7\,\mu$.) The definitive, but as yet faintly staining, yolk nucleus is present, and closely embraces the nucleus. Several nucleolar spherules stand in relation to the surface of the yolk nucleus, and in their spherical form offer a striking contrast to the granulo-reticular texture of both the yolk nucleus and general cytoplasm. $\times$ 1000.

Figs. 26 and 27.—Oocytes from sublimate material (nucleoli $= 8\,\mu$), at a slightly earlier stage to that shown in fig. 30, and after considerably greater extraction. Nuclear irregularity is as yet barely indicated. $\times$ 500.

Fig. 28.—Two adjacent oocytes from bichromate-acetic material. (Nucleoli $= 8\,\mu$.) In both the yolk nucleus is applied to the nucleus, but in the smaller egg it is faintly stained, and shows little or no indication of the clear space which accompanies the deeply stained yolk nucleus in the larger egg. $\times$ 500.

Fig. 29.—An oocyte from the same individual as figs. 26 and 27, but fixed in Hermann's fluid. (Nucleolus $= 8\,\mu$.) The definitive yolk nucleus is still applied to the nucleus, but is deeply stained, and a well marked clear space accompanies it on its outer side. On the side of the nucleus remote from the yolk nucleus is seen a compact area of yolk spherules; it is to this localised and precocious yolk formation that the irregular staining of the cytoplasm in this material after sublimate fixation is due (*e.g.*, fig. 27). $\times$ 500.

Figs. 30–33.—Oocytes at successive stages. (Nucleolus $= 10\,\mu$, $12\,\mu$, $14\,\mu$, $14\,\mu$), taken from sublimate material, to show the changes in form and position undergone by the yolk nucleus. The figures also show the marked irregularity of the nucleus always seen with this reagent at this period of the egg's growth. In figs. 30 and 31 the yolk nucleus is compact in form, measuring $25\,\mu$ in diameter and $5\,\mu$ in thickness, and stands midway between the nucleus and the egg-periphery. In fig. 32 yolk formation is just indicated; the yolk nucleus is already peripheral and measures $45\,\mu$ by $6\,\mu$, and in fig. 33, in which yolk formation is only slightly more advanced (the nucleolus still measuring $14\,\mu$), the yolk nucleus measures $100\,\mu$ in diameter by only $3\,\mu$ in thickness. In the last figure it can be seen that yolk formation commences in the yolk nucleus exactly as in other regions of the cytoplasm. $\times$ 500.

Fig. 34.—An oocyte from acetic-sublimate material (nucleolus $= 12\,\mu$), showing first trace of yolk formation. The yolk nucleus is compact and deeply stained, and not yet peripheral, and is completely surrounded by the clear space.

Note the strands of protoplasm still attached to the surface of the yolk nucleus. A portion of this egg is shown at a still greater magnification on Plate 31, fig. 62. $\times$ 500.

PLATE 30.

Figs. 35 and 36.—Two oocytes from the same section, Hermann material, to show the influence the contraction of the cytoplasm as a whole exerts on the size of the clear space accompanying the yolk nucleus. $\times$ 500.

Fig. 37.—An oocyte from Hermann material, showing a similar stage of yolk formation to that seen in fig. 33. (Nucleolus = 14 μ.) A few scattered peripheral spherules are present. $\times$ 500.

Fig. 38.—Oocyte at a stage of yolk formation slightly earlier than that seen in fig. 40, and taken from sublimate-acetic material. The peripheral spherules are numerous all over the cytoplasm, and are commencing to form a distinct peripheral layer. The chromatin spherules are commencing to appear on the as yet faintly staining chromatin of this material (*cf.* figs. 20, 22, 23 and 55 from same material). The vacuoles in the yolk nucleus indicate yolk formation, and are identical with those all over the cytoplasm, and are merely rendered more distinct by the greater staining capacity of the interspherular cytoplasm in this region. The correspondence between the outline of the yolk nucleus and the accompanying clear space indicates the origin of the latter by contraction during fixation. The figure shows well the entire absence of any spatial relation between the yolk nucleus and the peripheral spherules. $\times$ 500.

Fig. 39.—Oocyte from bichromate-acetic material (nucleolus = 14 μ), showing a very slightly earlier stage in yolk formation than that seen in fig. 41. The yolk nucleus in this egg is not discoid, but band-shaped and measures 140 μ by 40 μ by 3 μ.

Note the faintly stained condition of the cytoplasm with this reagent, although the yolk nucleus is deeply basophile. (*Cf.* also fig. 28.) $\times$ 500.

Fig. 40.—An oocyte taken from the same material as figs. 30–33, but representing a much later stage in yolk formation. (Nucleolus = 15 μ.) The yolk nucleus is now a faint and irregular area measuring 25 μ by 5 μ. This figure also clearly shows that yolk formation proceeds in the area of the yolk nucleus exactly as elsewhere. (*Cf.* also figs. 38 and 67.) The "peripheral spherules" are numerous all over the cytoplasm and are also commencing to form a distinct peripheral layer. (The relation between the group of the small cells at the surface of the egg and the more deeply staining perinuclear cytoplasm shown in this figure is referred to on p. 451.) $\times$ 500.

Fig. 41.—A slightly later stage in yolk formation to fig. 37, but from the same material. (Nucleolus = 14 μ.) The peripheral spherules are much more numerous, but there is still no peripheral layer. The yolk nucleus is already reduced to a faintly stained area at the periphery. (*Cf.* also fig. 40.)

Fig. 42.—A later stage in yolk formation from the same material as fig. 34. A portion of this egg at a still greater magnification is shown on Plate 31, fig. 63. Compare the condition of the chromatin and chromatin spherules at earlier (*e.g.*, fig. 62) and later (*e.g.*, 64) stages in the same material. $\times$ 500.

Figs. 43, 44, and 45 represent nucleoli from "BNO" material, and show the appearance of the "de-stained area" with this reagent. $\times$ 1000.

Fig. 46.—Nucleolus from Hermann material, showing a somewhat similar behaviour to that seen in "BNO" material; the region between the faintly staining internal area and the cortex showing a greater tenacity for the basic stain. $\times$ 1000.

Fig. 47.—Nucleolus from sublimate-acetic material showing the appearance presented by the last traces of the basophile substance. $\times$ 1000.

Figs. 48, 49, and 50.—Nucleoli from sublimate-acetic material, showing the appearance of the de-stained area frequently seen in the still growing oocyte. Fig. 50 also shows the relation of the nucleolar vacuoles to the cortical region. $\times$ 1000.

Fig. 51.—Shows the nucleolus in contact with the nuclear membrane by an extensive de-stained area, and very clearly indicates a more fluid consistency for the nucleolar substance in the absence of the basophile constituent. $\times$ 1000.

Fig. 52.—Nucleolus from adult egg in sublimate material. The de-stained area comes to the surface at three points, and to each a spherule in the caryolymph stands in spatial relation. A similar spherule is seen within a vacuole in the cortical substance of the nucleolus. $\times$ 1000.

Fig. 53.—A similar nucleolus from the same material. $\times$ 1000.

Fig. 54.—Nucleolus from adult egg in Hermann material. As in the last two figures, a deeply stained nucleolar spherule similar to those in the caryolymph is contained within a vacuole in the cortical substance of the nucleolus. The faintly stained condition of the nucleolus greatly accentuates the resemblance between the discharged nucleolar spherules and the one contained in the vacuole.

Note the evident distinction between the nucleolar spherules and those on the chromatin threads. $\times$ 1000.

Fig. 55.—Nucleolus from an adult egg, of the same material as figs. 20, 22, 23, 25, 36, and 38, and showing a very obvious discharge of "nucleolar spherules" from the de-stained area of the nucleolus. Unlike earlier stages in this

material, the chromatin is now deeply stained and distinctly spherular. × 1000.

Figs. 56–61.—Nucleoli from adult eggs in sublimate material, showing the relation of the discharged spherules to the de-stained area of the nucleolus, and the formation of the "nucleolar accumulation." × 1000.

Plate 31.

Figs. 62–64.—Represent four successive stages in yolk formation in sublimate-acetic material. Fig. 62 is an enlarged portion of the oocyte shown in fig. 34, while fig. 63 is a similar enlargement from fig. 42. Fig. 64, which is from the same material, represents a stage in yolk formation similar to that shown in fig. 40.

Note the gradual increase in the quantity and staining capacity of the chromatin, and in the size of the chromatin spherules, as yolk formation progresses. All × 2000.

Fig. 65.—Portion of adult egg from same material as preceding figures. Three "peripheral spherules" are seen stranded in the body of the yolk and offer a sharp contrast to the masses of slowly dissolving nucleolar spherules also present. × 2000.

Fig. 66.—Portion of adult egg from Hermann material. × 2000.

Note the great difference in the appearance of the chromatin spherules from that seen in the preceding figures, though the peripheral spherules are much the same.

Fig. 67.—A portion of the oocyte shown in fig. 40, but under a higher power, showing the course of yolk formation in the area of the yolk nucleus exactly as in the rest of the cytoplasm. × 2000.

Figs. 68 and 69.—Median and tangential sections of the same nucleus, to show the wrinkling of the nuclear membrane.

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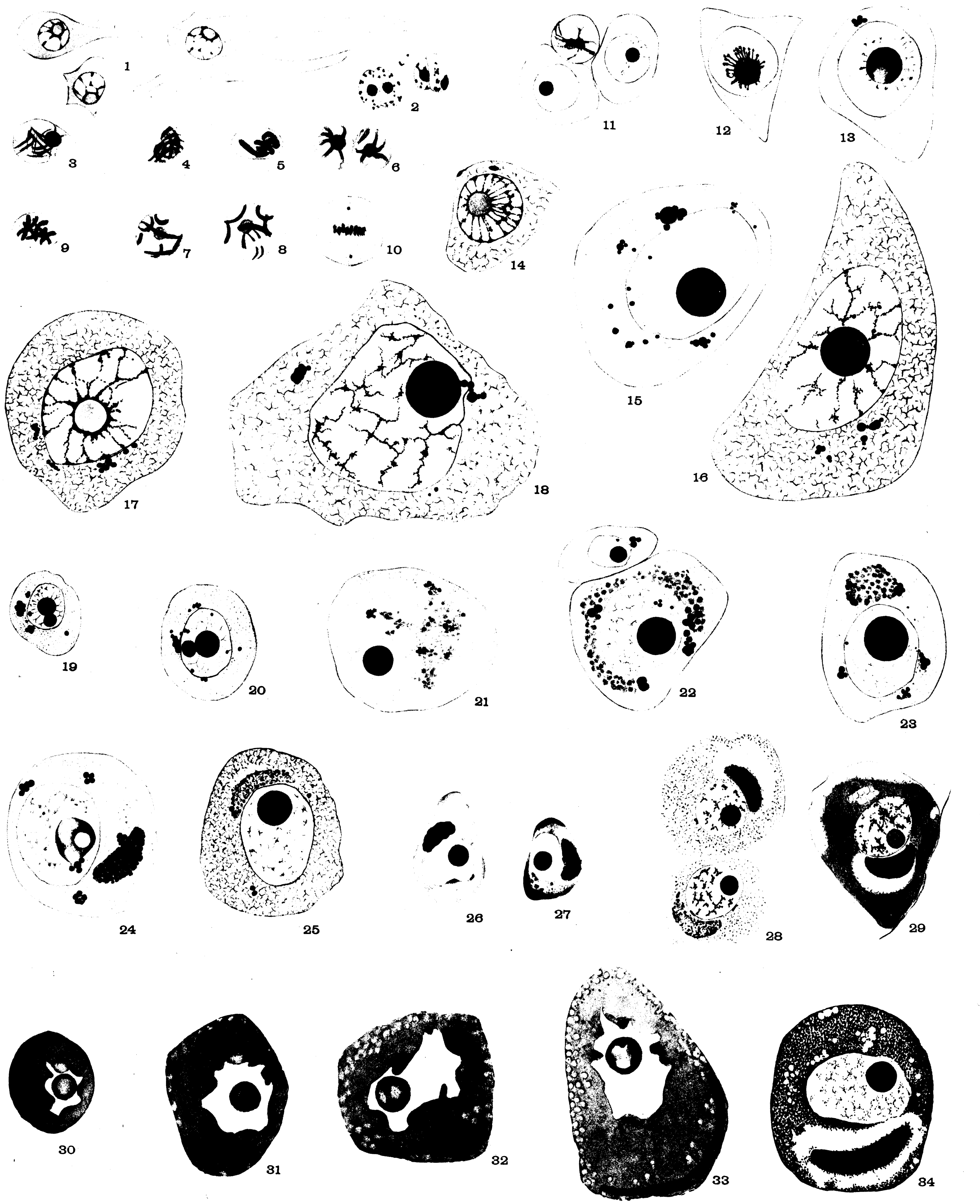

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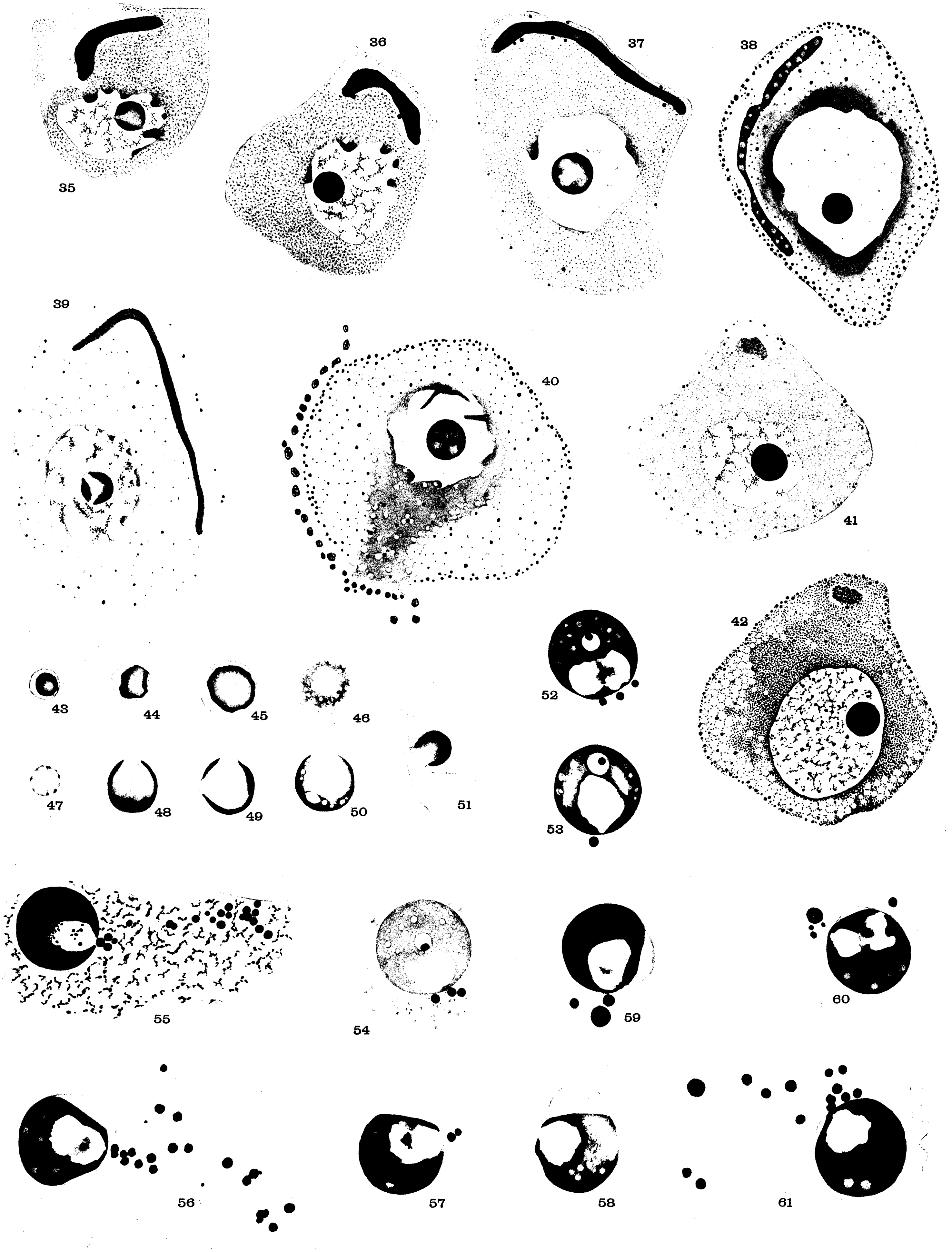

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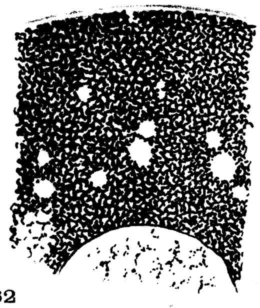

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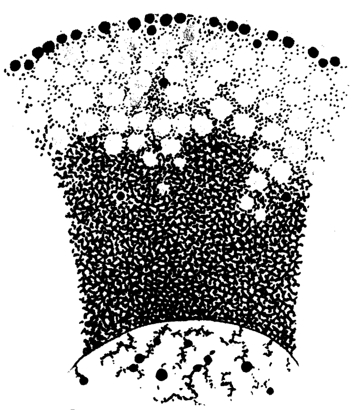

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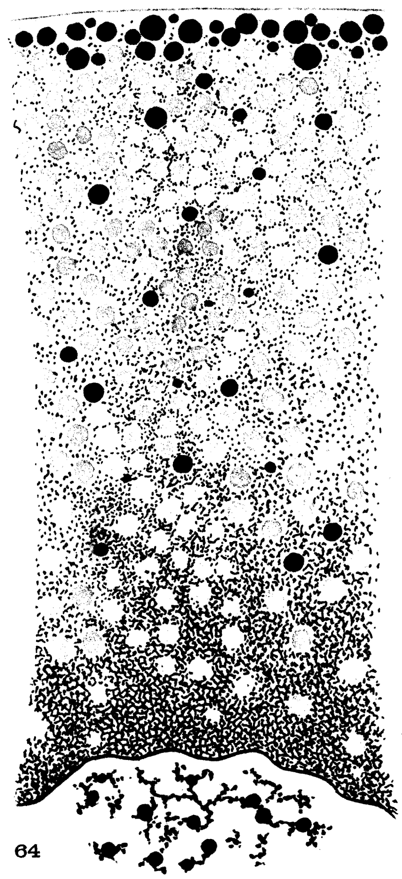

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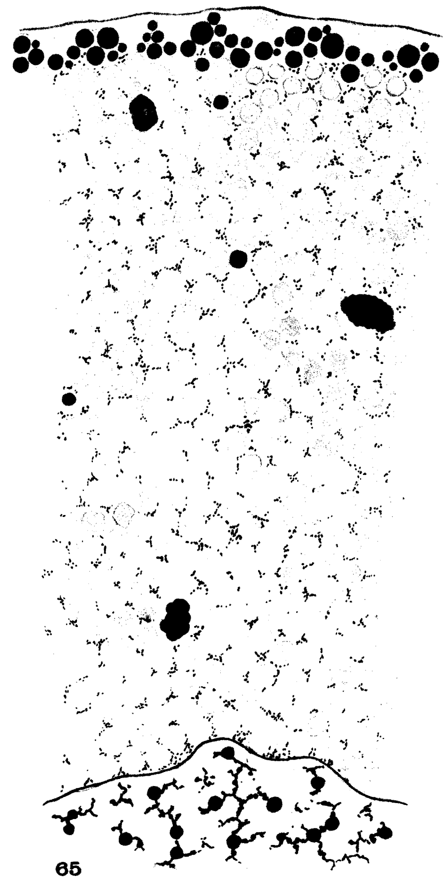

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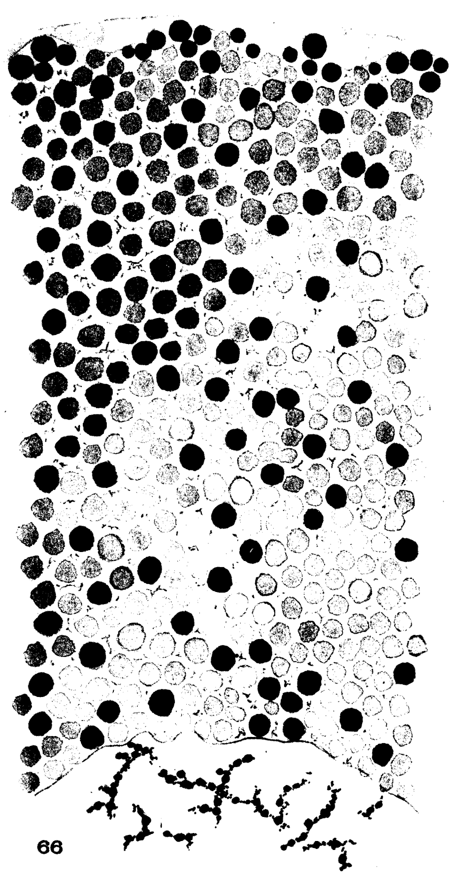

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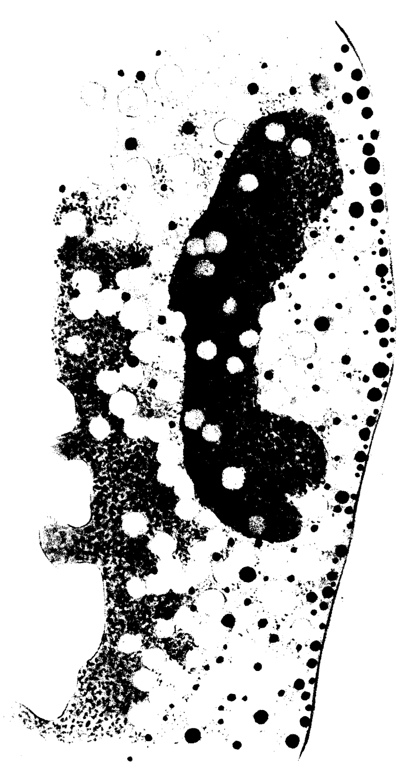

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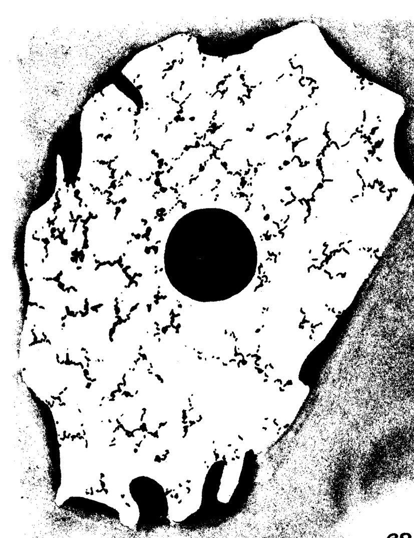

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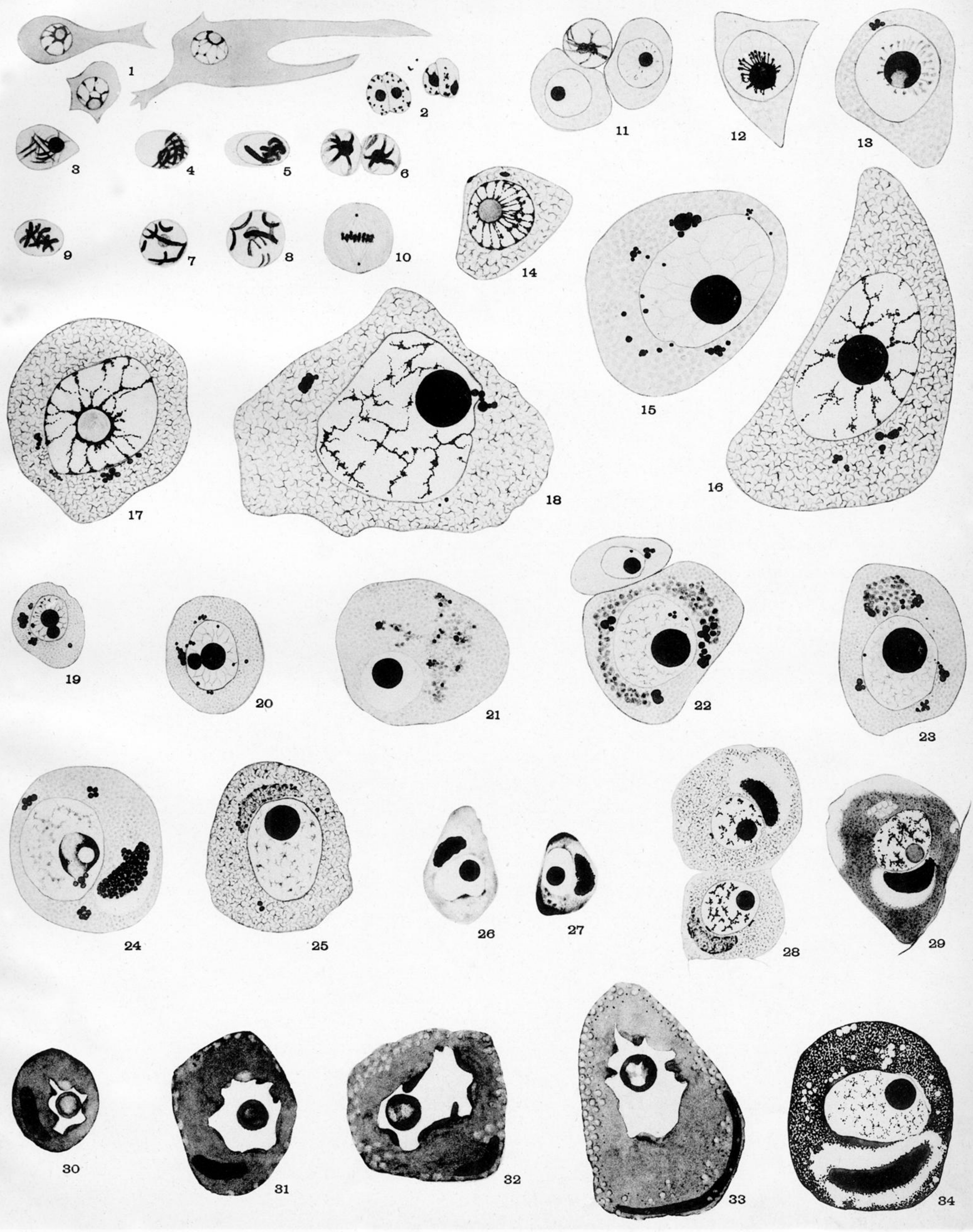


Plate 29.

Fig. 1.—Resting and freely floating oogonia from "BNO" material. The faintly stained nucleolus stands in sharp contrast to the enclosing chromatin. × 2000.

Fig. 2.—Double nuclei from a quite immature ovary. × 2000.

Fig. 3.—Early stage in synapsis, the split and segmented spireme thread is aggregating round the spherical and sharply-marked nucleolus. × 2000.

Fig. 4.—Synapsis stage; the longitudinal split is still distinctly visible. × 2000.

Fig. 5.—Synapsis stage; the longitudinal split is only doubtfully visible. × 2000.

Fig. 6.—Resolution of the synapsis; the nucleolus is again visible and perfectly normal in appearance. × 2000.

Figs. 7 and 8.—Formation of the definitive U-shaped chromosomes; the latter lie on the nuclear membrane. The nucleolus is central in position and still normal. In fig. 8 two of the chromosomes are still longitudinally split. × 2000.

Fig. 9.—Aggregation of the definitive chromosomes in the centre of the nucleus; the nucleolus is not visible at this stage. × 2000.

Fig. 10.—The completed mitotic figure. At this stage also there is no trace of the nucleolus. × 2000.

Fig. 11.—Two oocytes shortly after the completion of the oogonial mitosis. (Nucleus = 4·5 μ; nucleolus = 1·25 μ.) There is only a faint indication of a nuclear reticulum. An oogonial nucleus in prophase for mitosis is shown in close association. × 2000.

Fig. 12.—A somewhat older oocyte. (Nucleus = 7 μ; nucleolus = 2·5 μ.) The linin reticulum is distinctly visible, and the chromatin is commencing to extend out from the surface of the nucleolus. × 2000.

Fig. 13.—A similar stage in a slightly older oocyte. (Nucleolus = 3·5 μ.) The nucleolus shows an excentrically-placed de-stained area, and a group of "nucleolar" spherules is already present in the cytoplasm. × 2000.

Fig. 14.—Oocyte in which the chromatin extends as radial strands from the nucleolus to the nuclear membrane. (Nucleus = 7 μ; nucleolus = 2·5 μ.) × 2000.

Fig. 15.—Somewhat older oocyte than that shown in fig. 14. (Nucleus = 10 μ; nucleolus = 3·5 μ.) The (in this material) faintly stained chromatin is still radially arranged. Several groups of "nucleolar" spherules are present in the cytoplasm, and four spherules in the nucleus show a suggestive spatial relation to those in the cytoplasm. This figure shows well the spherical form, sharp outline, and variable size, of these nucleolar spherules. × 2000.

Fig. 16.—Slightly older oocyte, from bichromate-acetic material. (Nucleolus = 5 μ.) The chromatin is on the point of losing its connection, both with the surface of the nucleolus and with the nuclear membrane, and the strands are now distinctly granular. × 2000.

Fig. 17.—Oocyte from Hermann material. (Nucleolus = 3·5 μ.) Although the slide from which this section was taken was deeply stained, yet the nucleolus is faintly stained and stands in sharp contrast to the chromatin (*cf.* also fig. 54). Several groups of nucleolar spherules are present in the cytoplasm. × 2000.

Fig. 18.—Oocyte from bichromate-acetic material. (Nucleolus = 6 μ.) The chromatin is now in the form of scattered branched threads. The deeply stained and excentrically-placed nucleolus is seen in the act of discharging a stream of nucleolar spherules into the cytoplasm. A group of similar spherules is seen to the left. × 2000.

Figs. 19 and 20.—Oocytes showing double nucleolus. (Nucleolus = 3·5 μ and 2·5 μ : 2·5 μ and 5 μ). This condition is rarely met with at later stages. There are several groups of nucleolar spherules in the cytoplasm and a few in the nucleus. The chromatin is still radially arranged, and is (as in all but the older eggs of this particular material) faintly stained. (*Cf.* also figs. 22, 23, 38, and 55, all taken from the same material.) × 1000.

Fig. 21.—Oocyte from sublimate material. (Nucleolus = 6 μ.) The numerous nucleolar spherules in the cytoplasm are undergoing diffusion. × 1000.

Fig. 22.—Two adjacent oocytes. (Nucleoli = 3 μ and 7 μ.) In the older the diffusion of the nucleolar spherules is in progress, and the more recently discharged spherules offer a striking contrast to the more deeply stained cytoplasmic area to which this diffusion gives rise. (*Cf.* also previous figure.) The younger oocyte shows a group of nucleolar spherules in the cytoplasm which are almost as large as those seen in the larger oocyte. (*Cf.* also figs. 19, 15, etc.) × 1000.

Fig. 23.—Oocyte at a slightly later stage than the larger one in fig. 22. (Nucleolus = 8 μ.) The more deeply staining area of the cytoplasm is concentrating to form the definitive yolk nucleus, and there are also present three groups of as yet unaltered spherules. × 1000.

Fig. 24.—A similar oocyte (nucleolus = 8 μ), but in which the nucleolus is seen in the act of discharging a group of nucleolar spherules from the de-stained area. As in the preceding figure, several nucleolar spherules are seen in contact with the forming yolk nucleus. × 1000.

Fig. 25.—An oocyte from sublimate-acetic material. (Nucleolus = 7 μ.) The definitive, but as yet faintly staining, yolk nucleus is present, and closely embraces the nucleus. Several nucleolar spherules stand in relation to the surface of the yolk nucleus, and in their spherical form offer a striking contrast to the granulo-reticular texture of both the yolk nucleus and general cytoplasm. × 1000.

Figs. 26 and 27.—Oocytes from sublimate material (nucleoli = 8 μ), at a slightly earlier stage to that shown in fig. 30, and after considerably greater extraction. Nuclear irregularity is as yet barely indicated. × 500.

Fig. 28.—Two adjacent oocytes from bichromate-acetic material. (Nucleoli = 8 μ.) In both the yolk nucleus is applied to the nucleus, but in the smaller egg it is faintly stained, and shows little or no indication of the clear space which accompanies the deeply stained yolk nucleus in the larger egg. × 500.

Fig. 29.—An oocyte from the same individual as figs. 26 and 27, but fixed in Hermann's fluid. (Nucleolus = 8 μ.) The definitive yolk nucleus is still applied to the nucleus, but is deeply stained, and a well marked clear space accompanies it on its outer side. On the side of the nucleus remote from the yolk nucleus is seen a compact area of yolk spherules; it is to this localised and precocious yolk formation that the irregular staining of the cytoplasm in this material after sublimate fixation is due (*e.g.*, fig. 27). × 500.

Figs. 30–33.—Oocytes at successive stages. (Nucleolus = 10 μ, 12 μ, 14 μ, 14 μ), taken from sublimate material, to show the changes in form and position undergone by the yolk nucleus. The figures also show the marked irregularity of the nucleus always seen with this reagent at this period of the egg's growth. In figs. 30 and 31 the yolk nucleus is compact in form, measuring 25 μ in diameter and 5 μ in thickness, and stands midway between the nucleus and the egg-periphery. In fig. 32 yolk formation is just indicated; the yolk nucleus is already peripheral and measures 45 μ by 6 μ, and in fig. 33, in which yolk formation is only slightly more advanced (the nucleolus still measuring 14 μ), the yolk nucleus measures 100 μ in diameter by only 3 μ in thickness. In the last figure it can be seen that yolk formation commences in the yolk nucleus exactly as in other regions of the cytoplasm. × 500.

Fig. 34.—An oocyte from acetic-sublimate material (nucleolus = 12 μ), showing first trace of yolk formation. The yolk nucleus is compact and deeply stained, and not yet peripheral, and is completely surrounded by the clear space.

Note the strands of protoplasm still attached to the surface of the yolk nucleus. A portion of this egg is shown at a still greater magnification on Plate 31, fig. 62. × 500.

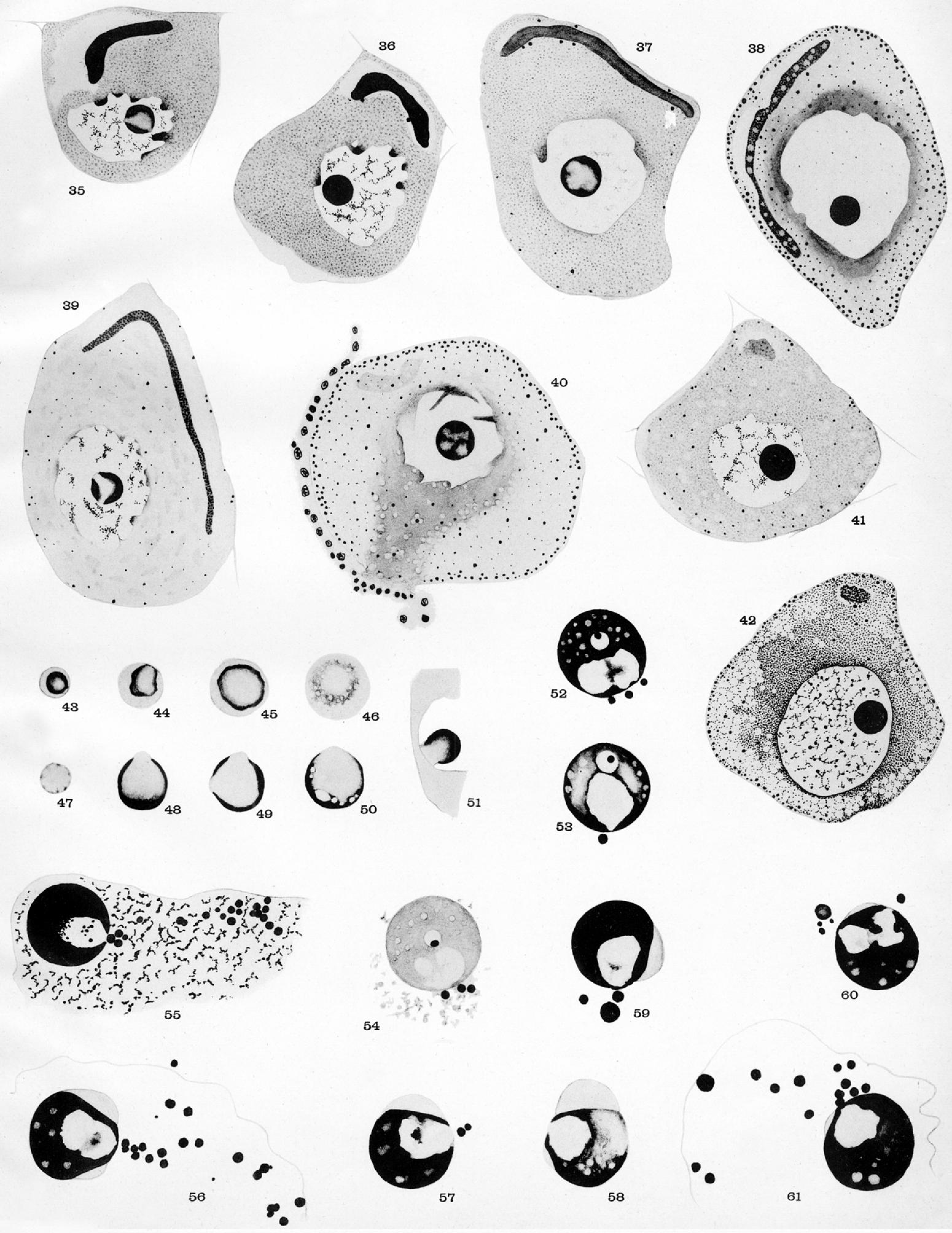


PLATE 30.

Figs. 35 and 36.—Two oocytes from the same section, Hermann material, to show the influence the contraction of the cytoplasm as a whole exerts on the size of the clear space accompanying the yolk nucleus. $\times$ 500.

Fig. 37.—An oocyte from Hermann material, showing a similar stage of yolk formation to that seen in fig. 33. (Nucleolus = 14 μ.) A few scattered peripheral spherules are present. $\times$ 500.

Fig. 38.—Oocyte at a stage of yolk formation slightly earlier than that seen in fig. 40, and taken from sublimate-acetic material. The peripheral spherules are numerous all over the cytoplasm, and are commencing to form a distinct peripheral layer. The chromatin spherules are commencing to appear on the as yet faintly staining chromatin of this material (*cf.* figs. 20, 22, 23 and 55 from same material). The vacuoles in the yolk nucleus indicate yolk formation, and are identical with those all over the cytoplasm, and are merely rendered more distinct by the greater staining capacity of the interspherular cytoplasm in this region. The correspondence between the outline of the yolk nucleus and the accompanying clear space indicates the origin of the latter by contraction during fixation. The figure shows well the entire absence of any spatial relation between the yolk nucleus and the peripheral spherules. $\times$ 500.

Fig. 39.—Oocyte from bichromate-acetic material (nucleolus = 14 μ), showing a very slightly earlier stage in yolk formation than that seen in fig. 41. The yolk nucleus in this egg is not discoid, but band-shaped and measures 140 μ by 40 μ by 3 μ.

Note the faintly stained condition of the cytoplasm with this reagent, although the yolk nucleus is deeply basophile. (*Cf.* also fig. 28.) $\times$ 500.

Fig. 40.—An oocyte taken from the same material as figs. 30–33, but representing a much later stage in yolk formation. (Nucleolus = 15 μ.) The yolk nucleus is now a faint and irregular area measuring 25 μ by 5 μ. This figure also clearly shows that yolk formation proceeds in the area of the yolk nucleus exactly as elsewhere. (*Cf.* also figs. 38 and 67.) The "peripheral spherules" are numerous all over the cytoplasm and are also commencing to form a distinct peripheral layer. (The relation between the group of the small cells at the surface of the egg and the more deeply staining perinuclear cytoplasm shown in this figure is referred to on p. 451.) $\times$ 500.

Fig. 41.—A slightly later stage in yolk formation to fig. 37, but from the same material. (Nucleolus = 14 μ.) The peripheral spherules are much more numerous, but there is still no peripheral layer. The yolk nucleus is already reduced to a faintly stained area at the periphery. (*Cf.* also fig. 40.)

Fig. 42.—A later stage in yolk formation from the same material as fig. 34. A portion of this egg at a still greater magnification is shown on Plate 31, fig. 63. Compare the condition of the chromatin and chromatin spherules at earlier (*e.g.*, fig. 62) and later (*e.g.*, 64) stages in the same material. $\times$ 500.

Figs. 43, 44, and 45 represent nucleoli from "BNO" material, and show the appearance of the "de-stained area" with this reagent. $\times$ 1000.

Fig. 46.—Nucleolus from Hermann material, showing a somewhat similar behaviour to that seen in "BNO" material; the region between the faintly staining internal area and the cortex showing a greater tenacity for the basic stain. $\times$ 1000.

Fig. 47.—Nucleolus from sublimate-acetic material showing the appearance presented by the last traces of the basophile substance. $\times$ 1000.

Figs. 48, 49, and 50.—Nucleoli from sublimate-acetic material, showing the appearance of the de-stained area frequently seen in the still growing oocyte. Fig. 50 also shows the relation of the nucleolar vacuoles to the cortical region. $\times$ 1000.

Fig. 51.—Shows the nucleolus in contact with the nuclear membrane by an extensive de-stained area, and very clearly indicates a more fluid consistency for the nucleolar substance in the absence of the basophile constituent. $\times$ 1000.

Fig. 52.—Nucleolus from adult egg in sublimate material. The de-stained area comes to the surface at three points, and to each a spherule in the caryolymph stands in spatial relation. A similar spherule is seen within a vacuole in the cortical substance of the nucleolus. $\times$ 1000.

Fig. 53.—A similar nucleolus from the same material. $\times$ 1000.

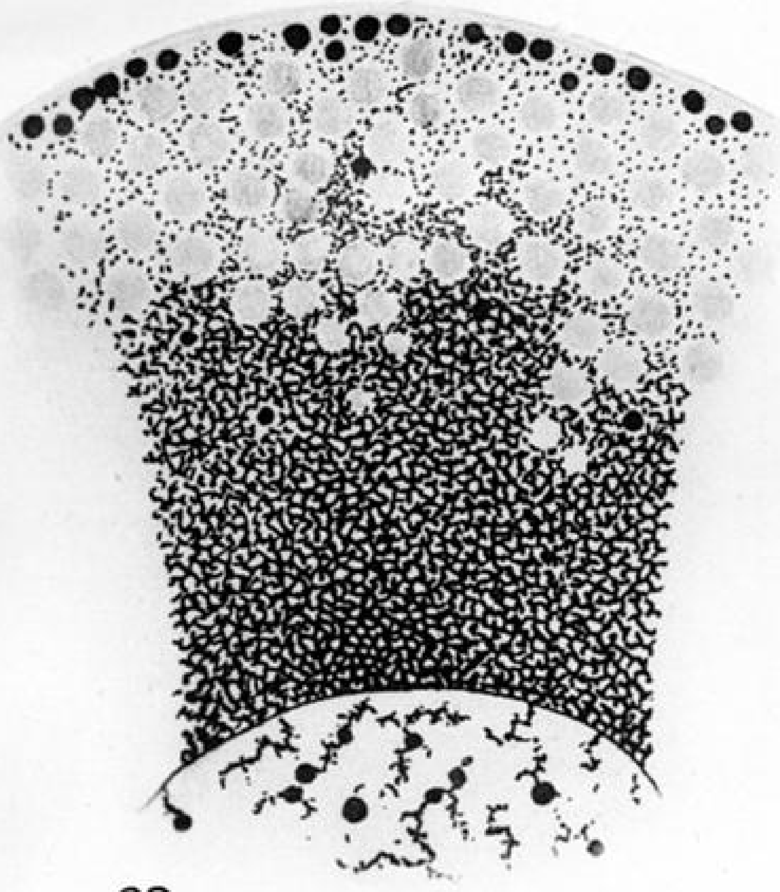
Fig. 54.—Nucleolus from adult egg in Hermann material. As in the last two figures, a deeply stained nucleolar spherule similar to those in the caryolymph is contained within a vacuole in the cortical substance of the nucleolus. The faintly stained condition of the nucleolus greatly accentuates the resemblance between the discharged nucleolar spherules and the one contained in the vacuole.

Note the evident distinction between the nucleolar spherules and those on the chromatin threads. $\times$ 1000.

Fig. 55.—Nucleolus from an adult egg, of the same material as figs. 20, 22, 23, 25, 36, and 38, and showing a very obvious discharge of "nucleolar spherules" from the de-stained area of the nucleolus. Unlike earlier stages in this material, the chromatin is now deeply stained and distinctly spherular. $\times$ 1000.

Figs. 56–61.—Nucleoli from adult eggs in sublimate material, showing the relation of the discharged spherules to the de-stained area of the nucleolus, and the formation of the "nucleolar accumulation." $\times$ 1000.

62



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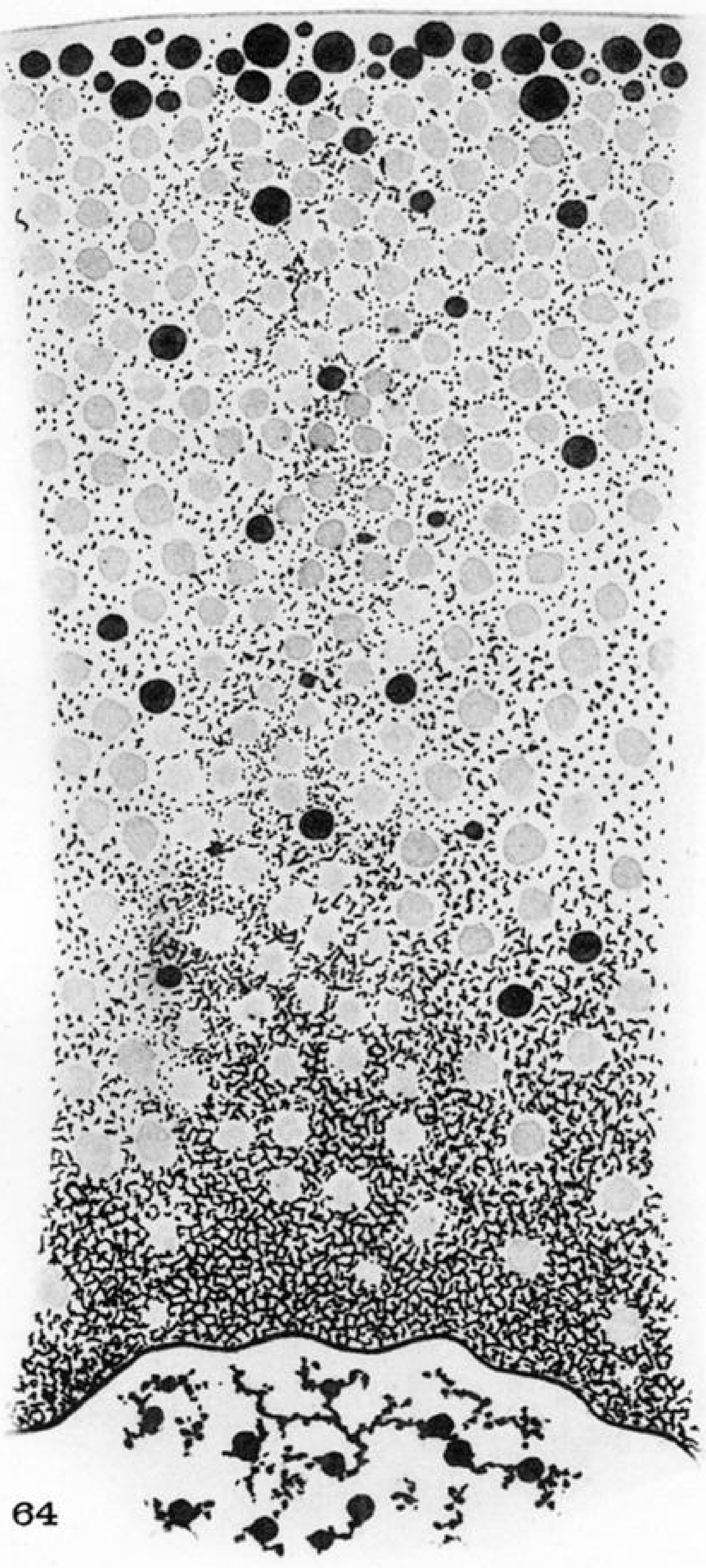

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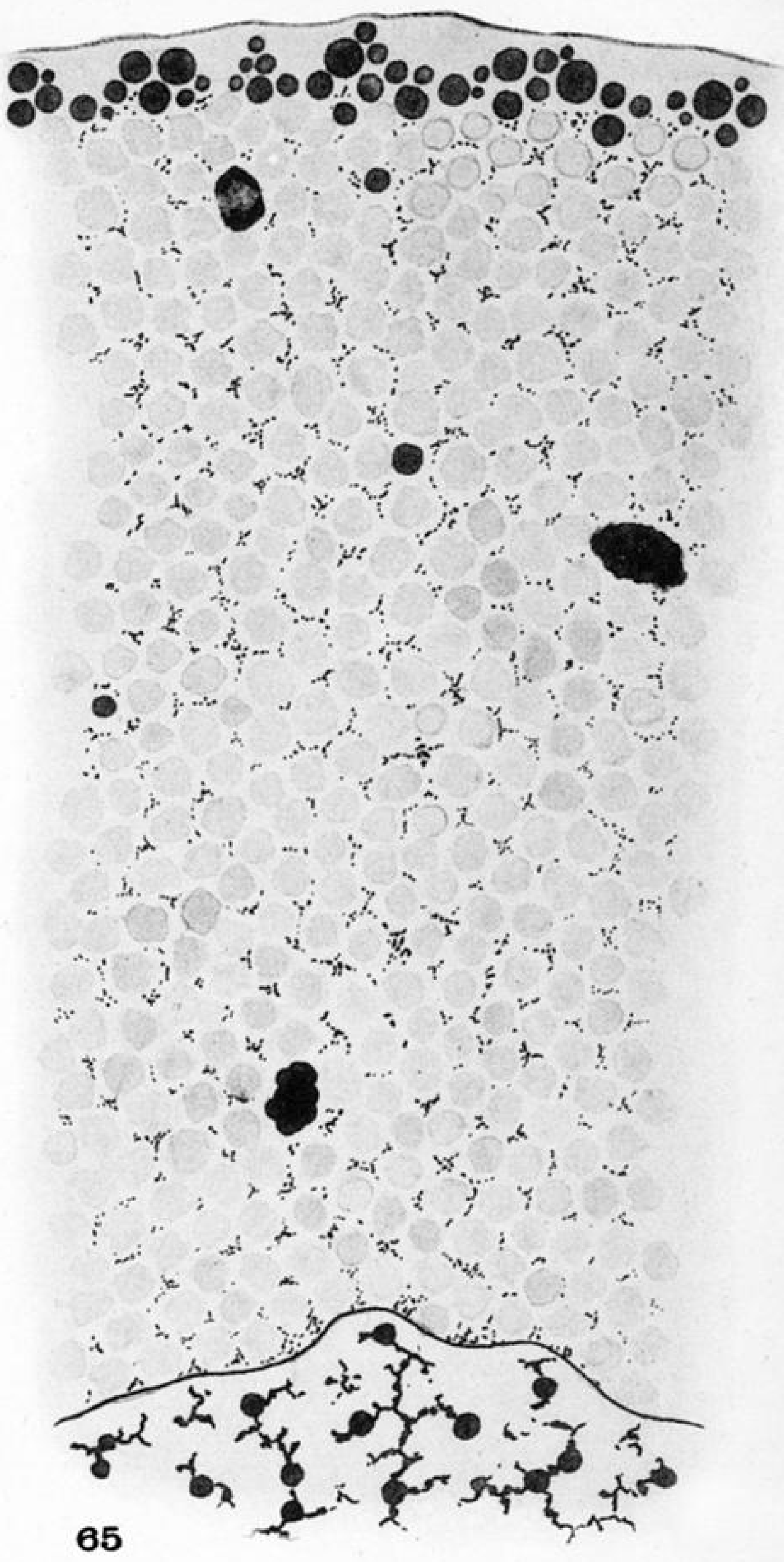

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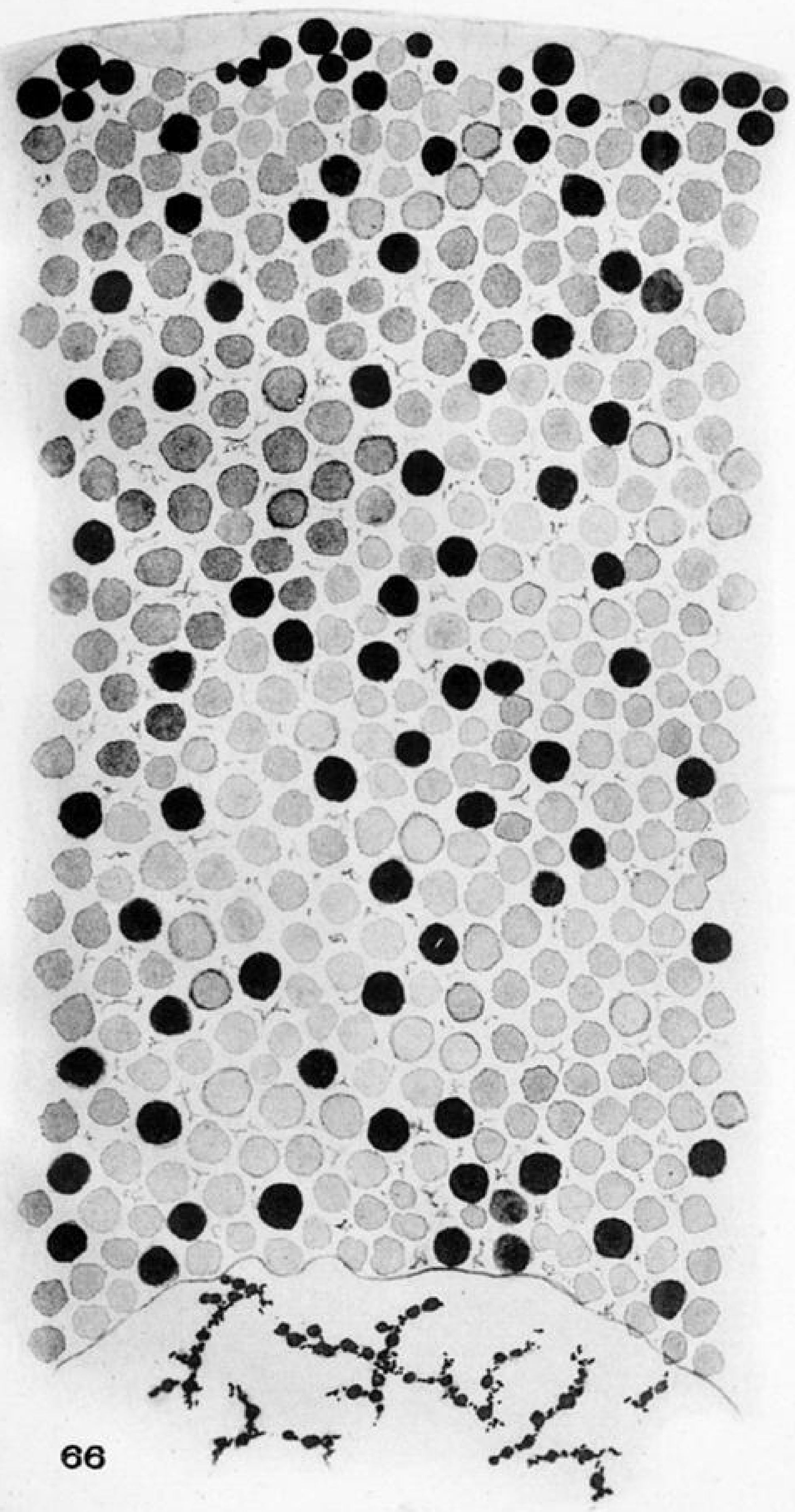

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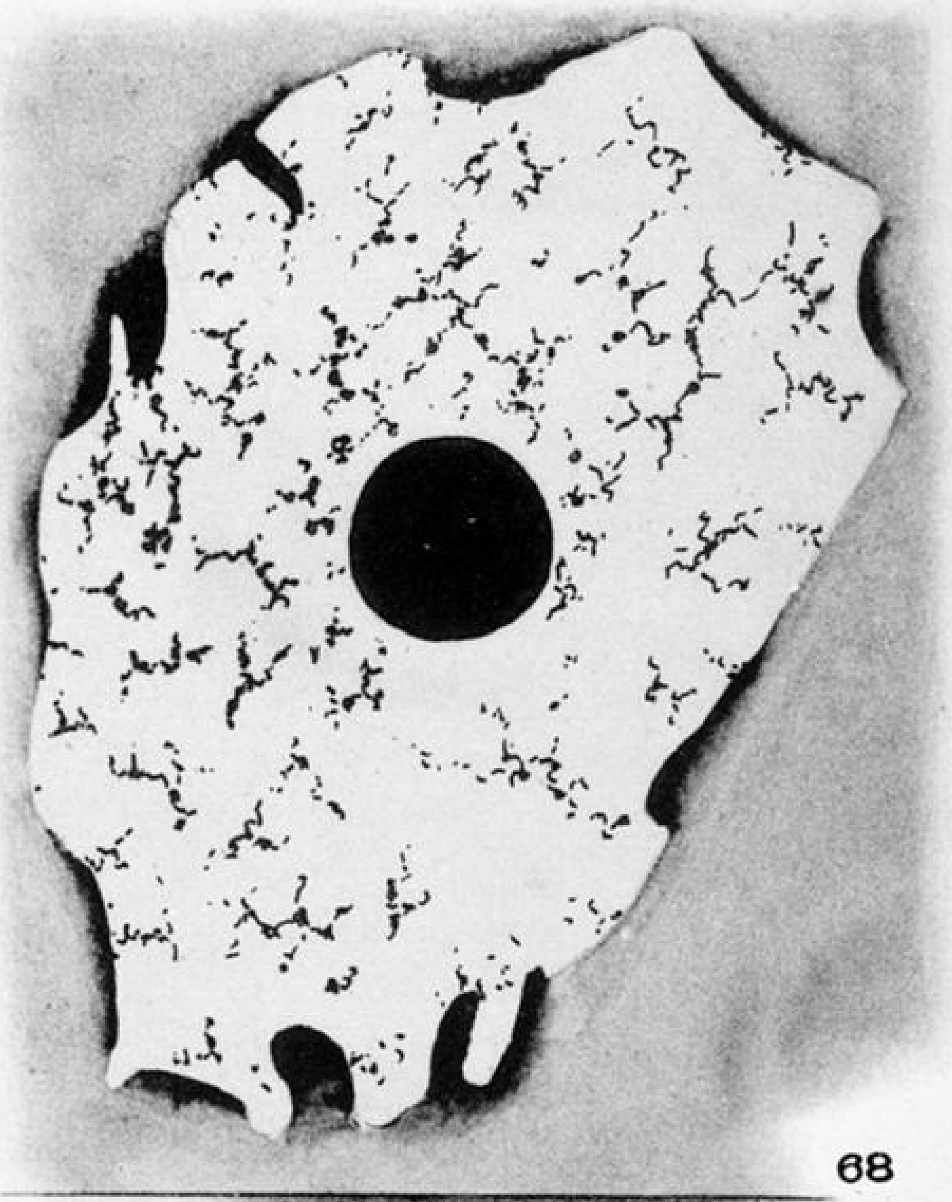

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PLATE 31.

Figs. 62–64.—Represent four successive stages in yolk formation in sublimate-acetic material. Fig. 62 is an enlarged portion of the oocyte shown in fig. 34, while fig. 63 is a similar enlargement from fig. 42. Fig. 64, which is from the same material, represents a stage in yolk formation similar to that shown in fig. 40.

Note the gradual increase in the quantity and staining capacity of the chromatin, and in the size of the chromatin spherules, as yolk formation progresses. All × 2000.

Fig. 65.— Portion of adult egg from same material as preceding figures. Three "peripheral spherules" are seen stranded in the body of the yolk and offer a sharp contrast to the masses of slowly dissolving nucleolar spherules also present. × 2000.

Fig. 66.—Portion of adult egg from Hermann material. × 2000.

Note the great difference in the appearance of the chromatin spherules from that seen in the preceding figures, though the peripheral spherules are much the same.

Fig. 67.—A portion of the oocyte shown in fig. 40, but under a higher power, showing the course of yolk formation in the area of the yolk nucleus exactly as in the rest of the cytoplasm. × 2000.

Figs. 68 and 69.—Median and tangential sections of the same nucleus, to show the wrinkling of the nuclear membrane.